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(57) Abstract

Improved methods for inhibiting rejection of transplanted cells in allogeneic or xenogeneic recipient subject are described. The methods involve altering at least one antigen on the surface of a donor cell prior to transplantation to reduce the immunogenicity of the cell in a recipient subject. Preferably, an MHC class I antigen on a donor cell is altered by contacting the cell with a molecule which binds to the antigen, such as an antibody or fragment or derivative thereof. The altered cell can then be transplanted into a recipient subject such that immune cell-mediated, e.g., T cell-mediated, NK cell-mediated, and/or LAK cell-mediated, rejection is inhibited.

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MODIFIED CELLS AND METHODS FOR INHIBITING XENOGRAFT REJECTION

Background of the Invention

A number of diseases are treated by the transplantation of tissue donated by other human (allografts) or obtained from animals (xenografts). Examples of such diseases include Parkinson's disease, which can be treated by transplantation of neural cells, and insulin-dependent diabetes, which can be treated by transplantation of insulin-secreting pancreatic islet cells. While the transplanted cells may have the capacity to perform the desired function (e.g., secretion of insulin in response to the rising levels of glucose), the graft will soon fail as a result of immunological rejection. Shortly after transplantation, cells of the immune system of the recipient recognize the allogeneic or xenogeneic cells as foreign and proceed to attack the graft through both humoral and cellular routes. Allogeneic or xenogeneic cells are initially recognized by the recipient's immune system through antigenic determinants expressed on the surface of the cells. The predominant antigens recognized as "non-self" are the major histocompatibility complex class I and class II antigens (MHC class I and class II). MHC class I antigens are expressed on virtually all parenchymal cells (e.g., pancreatic islet cells). In contrast, MHC class II antigens are expressed on a limited number of cell types, primarily B cells, macrophages, dendritic cells, Langerhans cells and thymic epithelium. The interaction of foreign MHC antigens with the T cell receptor on host T cells causes these host cells to become activated. Following activation, these T cells proliferate and induce effector functions which lead to cell lysis and destruction of the transplanted cells.

For transplantation to be a viable therapeutic option, approaches are needed to inhibit rejection of transplanted cells by the immune system of the recipient. One method for inhibiting this rejection process is by administration of drugs that suppress the function of the immune system. Drugs such as cyclophosphamide and cyclosporin can inhibit the actions of the immune system and thus allow graft acceptance. However, these drugs generally need to be administered to a graft recipient permanently (i.e., life-long) and their use results in generalized immunosuppression which leaves the recipient susceptible to infection and tumor growth. Additionally, administration of immunosuppressive drugs is often accompanied by other serious side effects such as renal failure and hypertension. The requirement for life-long administration of immunosuppressive drugs in transplant recipients illustrates the need for better methods for transplanting cells such that rejection of the cells by the recipient's immune system is inhibited.

It has been shown that it is possible to alter an antigen on the surface of a cell to be transplanted prior to transplantation to "mask" the antigen from normal recognition by cells of the recipient's immune system (see Faustman & Coe (1991) *Science* 252:1700-1702 and WO 92/04033). For example, MHC class I antigens on transplanted cells can be altered by contacting the cells with a molecule which binds to the antigen, such as an antibody or

fragment thereof (e.g., a F(ab')$_2$ fragment) prior to transplantation. This alteration of MHC class I antigens modifies the interaction between the antigens on the cells and immune cells in the recipient following transplantation, to thereby inhibit rejection of the transplanted cells. Additional methods for inhibiting rejection of an allograft or xenograft following transplantation in a host are needed.

Summary of the Invention

This invention pertains to methods for transplanting cells into an allogeneic or xenogeneic recipient such that rejection of the cells by the recipient is inhibited. The methods of the invention involve modification of donor cells prior to transplantation to reduce the immunogenicity of the cells in a recipient. In the preferred embodiment, this invention features treatment of donor cells to modify surface antigens prior to transplantation such that upon transplantation into a recipient subject natural killer (NK) cell-mediated rejection and/or lymphokine activated killer (LAK) cell-mediated rejection of the cell is inhibited. As a result of this treatment, rejection of donor cells in the recipient is inhibited.

The present invention pertains to a cell (i.e., a donor cell) which has at least one antigen on the cell surface which stimulates an immune response against the cell when the cell is transplanted into a recipient subject, for example, a xenogeneic subject. The antigen on the surface of the cell is altered such that rejection of the cell is inhibited. Alteration of the cell surface antigen can inhibit rejection of the cell by a variety of mechanisms. For example, alteration of the antigen can modify an interaction between the antigen and a an immune cell such as a lymphocyte, e.g., a T lymphocyte, a B lymphocyte, a natural killer cell, or a lymphokine activated killer cell, in the recipient, thereby inhibiting an immune response against the cell in the recipient. The antigen(s) on the surface of the cells to be altered is one which is capable of stimulating an immune response against the cell in the recipient. An antigen on the surface of a cell can be altered prior to transplantation by contacting the cell *in vitro* with a molecule which binds to the antigen. In one embodiment, the molecule which binds to the antigen is an antibody, or fragment or derivative thereof, which binds to the antigen but does not activate complement or induce lysis of the cell. A preferred antibody fragment is an F(ab')$_2$ fragment. Alternatively, the molecule is a peptide or derivative thereof (e.g., a peptide mimetic) which binds the antigen and interferes with an interaction with an immune cell. In a preferred embodiment, the antigen on the cell surface which is altered is an MHC class I antigen. Preferred antibodies which can be used to alter MHC class I antigens on the surface of cells include the monoclonal antibodies W6/32 and PT85, or fragments or derivatives thereof, or other antibodies which bind to the same epitopes recognized by the W6/32 and PT85 antibodies. Other cell surface antigens which can be altered include adhesion molecules, such as ICAM-1, ICAM-2 and LFA-3.

Preferred cells of the invention are porcine cells. The porcine cells can be endothelial cells, hepatocytes, pancreatic islet cells, skeletal myocytes, skeletal myoblasts, cardiac

myocytes, cardiac myoblasts, fibroblasts, epithelial cells, neural cells, e.g., mesencephalic cells, striatal cells, cortical cells, bone marrow cells, hematopoietic cells, and lymphoid cells. The cells can be within a tissue or an organ.

Another aspect of the present invention is a method for reducing the immunogenicity of a cell for transplantation into a recipient subject. This method includes contacting a cell which has at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject with at least one molecule which binds to the antigen on the cell surface. When such a cell is transplanted into recipient subject, natural killer cell-mediated rejection and/or lymphokine activated killer cell-mediated rejection of the cell is inhibited. Preferred antigens and molecules which bind such antigens are described herein. Preferred recipient subjects include humans.

A further aspect of the present invention is a method for transplanting a cell into a recipient subject such that rejection of the cell by the recipient subject, e.g., a xenogeneic subject, is inhibited. This method includes administering to the subject a cell having at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject, wherein the at least one antigen on the cell surface is altered prior to transplantation to inhibit natural killer cell-mediated rejection and/or lymphokine activated killer cell-mediated rejection of the cell by the recipient subject. Preferred antigens, molecules which bind such antigens, and donor cells are described herein.

In addition to inhibiting rejection of transplanted cells, the methods of the invention induce donor cell-specific T cell tolerance or nonresponsiveness to the transplanted cells in the transplant recipient. The invention thus provides methods for successful transplantation of cells into an allogeneic or xenogeneic transplant recipient which avoids life-long generalized immunosuppression of the subject.

Brief Description of the Drawings

Figure 1 shows a graph depicting the results of a cytolytic assay in which freshly isolated human peripheral blood lymphocytes (PBL) were shown to lyse porcine PBLs.

Figure 2 shows a graph depicting the results of a cytolytic assay in which freshly isolated human PBLs were shown to lyse porcine hepatocytes.

Figure 3 shows a graph depicting the results of a mixed lymphocyte reaction (MLR) in which untreated porcine kidney cells or porcine kidney cells treated with anti-MHC Class I antibody PT85 $F(ab')_2$ fragments were incubated with human PBLs. Human PBL proliferation in response to the treated porcine kidney cells was reduced compared to the human PBL proliferation in response to the untreated porcine kidney cells.

Figure 4 shows the results of a FACS analysis of human cells isolated from an MLR (the results of the MLR are described for Figure 3) and stained with monoclonal antibodies for CD56. When human PBLs were stimulated with untreated porcine kidney cells, the FACS staining revealed a remarkable increase in human cells expressing CD56 (NCAM,

which is a marker for natural killer (NK) cells). However, when human PBLs were stimulated with porcine kidney cells treated with anti-MHC Class I antibody PT85 $F(ab')_2$ fragments, there was not a substantial increase in CD56 expressing cells.

Figures 5A-5C show the results of a ^{51}Cr release assay in which freshly isolated PBLs from three individuals (A, B, and C) were used as effector cells against porcine PBLs in the presence of pooled human serum (•); serum-free media (◊); JY target cells (serum free) (o). The ^{51}Cr release assay revealed that freshly isolated human PBLs lysed porcine cells but not human cells.

Figure 6 shows the results of a ^{51}Cr release assay in which human PBLs cultured for 6 days with *aa* haplotype porcine PBLs were used as effectors against porcine PBLs. Target cells were as follows: *aa* target cells (•); *dd* target cells (◊); JY target cells (o). The ^{51}Cr release assay revealed that human PBLs, after culture with porcine cells, lysed porcine cells regardless of MHC restriction.

Figures 7A-7B show the results of a ^{51}Cr release assay in which K562 or JY cells were used as cold target inhibitors against porcine PBLs. In Figure 7A, freshly isolated human PBLs were used as effectors while in Figure 7B effector cells were harvested from a 6 day mixed culture of human PBLs and porcine PBLs. Inhibitors were as follows: no inhibitors (•); JY inhibitors (◊); K562 inhibitors (o). The ^{51}Cr release assay revealed that K562 cells inhibit lysis of porcine cells when freshly isolated human PBLs are used as effectors and JY cells do not inhibit lysis of porcine cells when freshly isolated human PBLs are used as effectors.

Figures 8A-8C show the results of a ^{51}Cr release assay in which unfractionated human PBLs (Figure 8A), CD56 enriched PBLs (Figure 8B), and CD56 depleted cells (Figure 8C) were used as human effector cells against porcine PBLs. Target cells were as follows: K562 cells (•); porcine PBLs (◊). The ^{51}Cr release assay revealed that most of the cytotoxic activity toward porcine cells and K562 cells is present in the CD56-enriched population and not in the CD56-depleted population.

Figures 9A-9B show the results of a ^{51}Cr release assay in which freshly isolated human PBLs (Figure 9A) and human PBLs previously cultured with mitomycin C-treated porcine PBLs for 6 days (Figure 9B) were used as effectors against porcine PBLs. Inhibitors were as follows: no cold target inhibitors (•); K562 inhibitors (◊); Daudi inhibitors (o). The ^{51}Cr release assay revealed that while K562 cells inhibited the unstimulated human anti-porcine cytotoxicity as well as cytotoxicity after mixed culture, Daudi cells inhibited cytotoxicity after mixed culture only.

Figure 10 shows the results of an ELISA in which the supernatant of mixed cultures of human PBLs and mitomycin-C-treated porcine PBLs was tested for IL-2 production. Black bars, no antibody added; Hatched bars, anti-CD25 added. The ELISA showed that human IL-2 is generated in these cultures.

Figure 11 shows the results of a ^{51}Cr release assay in which human PBLs, previously cultured with mitomycin C-treated porcine PBLs for 6 days, were used as effectors against porcine PBLs. No antibody added (•); control IgG added (o); anti-CD25 added (◊). The ^{51}Cr release assay revealed that anti-CD25 antibody blocks generation of anti-porcine human cytotoxic cells.

Figure 12 shows the results of a ^{51}Cr release assay in which a CD56-depleted population of human PBLs were used as effectors against porcine PBLs. This population was generated by depleting $CD56^+$ cells from human PBL preparations, culturing the cells for 6 days with mitomycin C-treated porcine PBLs, and repeating the $CD56^+$ cell depletion. A: % specific lysis by CD56-depleted population; B: % specific lysis by CD56-depleted population in the presence of anti-CD3; C: % specific lysis by CD56-depleted population in the presence of control IgG; D: % specific lysis by CD56-depleted population in the presence of K562 cold target inhibitors; E: % specific lysis by CD56-depleted population in the presence of JY cold target inhibitors. The ^{51}Cr release assay demonstrated that, after depletion of $CD56^+$ cells, a T cell component of human anti-porcine cytotoxicity was apparent.

Detailed Description of the Invention

The invention features cells and methods for transplanting cells into an allogeneic or xenogeneic recipient such that rejection of transplanted cells by the recipient is inhibited. The cells to be transplanted into a recipient are treated such that at least one antigen on the surface of the cell is altered prior to transplantation to modify an interaction between the antigen and an immune cell (e.g., a natural killer cell, a lymphokine activated killer cell) in the recipient, thereby inhibiting rejection of the cells by the recipient. In addition to administration of the modified cell, the recipient can be treated with an agent which inhibits T cell activity in the recipient to further inhibit rejection of the transplanted cells.

The cells and methods of the invention are described in further detail in the following subsections.

I. Cells for Transplantation

One aspect of the invention relates to a modified or altered cell suitable for transplantation. Preferred cells of the invention are porcine cells, such as embryonic porcine cells. The porcine cells can be endothelial cells, hepatocytes, pancreatic islet cells, skeletal myocytes, skeletal myoblasts, cardiac myocytes, cardiac myoblasts, fibroblasts, epithelial cells, neural cells, e.g. mesencephalic cells, striatal cells, or cortical cells, bone marrow cells, hematopoietic cells, and lymphoid cells. The cells can be isolated or within a tissue or an organ.

In an unmodified or unaltered state, the antigen on the cell surface stimulates an immune response against the cell (also referred to herein as the donor cell) when the cell is administered to a subject (also referred to herein as the recipient, host, or recipient subject).

By altering the antigen, the normal immunological recognition of the donor cell by the immune system cells of the recipient is disrupted and additionally, "abnormal" immunological recognition of this altered form of the antigen can lead to donor cell-specific long term unresponsiveness in the recipient. Thus, alteration of an antigen on the donor cell prior to administering the cell to a recipient interferes with the initial phase of recognition of the donor cell by the cells of the host's immune system subsequent to administration of the cell. Furthermore, alteration of the antigen can induce immunological nonresponsiveness or tolerance, thereby preventing the induction of the effector phases of an immune response (e.g., cytotoxic T cell generation, antibody production etc.) which are ultimately responsible for rejection of foreign cells in a normal immune response. As used herein, the terms "altered" and "modified" are used interchangeably and encompasses changes that are made to a donor cell antigen which reduce the immunogenicity of the antigen to thereby interfere with immunological recognition of the antigen by the recipient's immune system. Preferably immunological nonresponsiveness to the donor cells in the recipient subject is generated as a result of alteration of the antigen. The terms "altered" and "modified" are not intended to include complete elimination of the antigen on the donor cell since delivery of an inappropriate or insufficient signal to the host's immune cells may be necessary to achieve immunological nonresponsiveness.

Antigens to be altered according to the invention include antigens on a donor cell which can interact with an immune cell (e.g., a hematopoietic cell, an NK cell, an LAK cell) in an allogeneic or xenogeneic recipient and thereby stimulate a specific immune response against the donor cell in the recipient. The interaction between the antigen and the immune cell may be an indirect interaction (e.g., mediated by soluble factors which induce a response in the hematopoietic cell, e.g., humoral mediated) or, preferably, is a direct interaction between the antigen and a molecule present on the surface of the immune cell (i.e., cell-cell mediated). As used herein, the phrase "immune cell" is intended to include hematopoietic cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, dendritic cells, and other antigen presenting cells, NK cells, and LAK cells. In preferred embodiments, the antigen is one which interacts with a T lymphocyte in the recipient (e.g., the antigen normally binds to a receptor on the surface of a T lymphocyte), or with an NK cell or LAK cell in the recipient.

In a preferred embodiment, the antigen on the donor cell to be altered is an MHC class I antigen. MHC class I antigens are present on almost all cell types. In a normal immune response, self MHC molecules function to present antigenic peptides to a T cell receptor (TCR) on the surface of self T lymphocytes. In immune recognition of allogeneic or xenogeneic cells, foreign MHC antigens (most likely together with a peptide bound thereto) on donor cells are recognized by the T cell receptor on host T cells to elicit an immune response. In addition, foreign MHC class I antigens are known to be recognized by MHC class I receptors on NK cells. MHC class I antigens on a donor cell are altered to interfere

with their recognition by T cells, NK cells, or LAK cells in an allogeneic or xenogeneic host (e.g., a portion of the MHC class I antigen which is normally recognized by the T cell receptor, NK cells, or LAK cells is blocked or "masked" such that normal recognition of the MHC class I antigen can no longer occur). Additionally, an altered form of an MHC class I antigen which is exposed to host T cells, NK cells or LAK cells (i.e., available for presentation to the host cell receptor) may deliver an inappropriate or insufficient signal to the host T cell such that, rather than stimulating an immune response against the allogeneic or xenogeneic cell, donor cell-specific T cell non-responsiveness, inhibition of NK-mediated cell rejection, and/or inhibition of LAK-mediated cell rejection is induced. For example, it is known that T cells which receive an inappropriate or insufficient signal through their T cell receptor (e.g., by binding to an MHC antigen in the absence of a costimulatory signal, such as that provided by B7) become anergic rather than activated and can remain refractory to restimulation for long periods of time (*see, e.g.*, Damle et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:5096-5100; Lesslauer et al. (1986) *Eur. J. Immunol.* 16:1289-1295; Gimmi, et al. (1991) *Proc. Natl. Acad. Sci. USA* 88: 6575-6579; Linsley et al. (1991) *J. Exp. Med.* 173:721-730; Koulova et al. (1991) *J. Exp. Med.* 173:759-762; Razi-Wolf, et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:4210-4214).

Alternative to MHC class I antigens, the antigen to be altered on a donor cell can be an MHC class II antigen. Similar to MHC class I antigens, MHC class II antigens function to present antigenic peptides to a T cell receptor on T lymphocytes. However, MHC class II antigens are present on a limited number of cell types (primarily B cells, macrophages, dendritic cells, Langerhans cells and thymic epithelial cells). In addition to or alternative to MHC antigens, other antigens on a donor cell which interact with molecules on host T cells or NK cells and which are known to be involved in immunological rejection of allogeneic or xenogeneic cells can be altered. Other donor cell antigens known to interact with host T cells and contribute to rejection of a donor cell include molecules which function to increase the avidity of the interaction between a donor cell and a host T cell. Due to this property, these molecules are typically referred to as adhesion molecules (although they may serve other functions in addition to increasing the adhesion between a donor cell and a host T cell). Examples of preferred adhesion molecules which can be altered according to the invention include LFA-3 and ICAM-1. These molecules are ligands for the CD2 and LFA-1 receptors, respectively, on T cells. By altering an adhesion molecule on the donor cell, (such as LFA-3, ICAM-1 or a similarly functioning molecule), the ability of the host's T cells to bind to and interact with the donor cell is reduced. Both LFA-3 and ICAM-1 are found on endothelial cells found within blood vessels in transplanted organs such as kidney and heart. Altering these antigens can facilitate transplantation of any vascularized implant, by altering recognition of those antigens by CD2+ and LFA-l+ host T-lymphocytes.

The presence of MHC molecules or adhesion molecules such as LFA-3, ICAM-1 etc. on a particular donor cell can be assessed by standard procedures known in the art. For

example, the donor cell can be reacted with a labeled antibody directed against the molecule to be detected (e.g., MHC molecule, ICAM-1, LFA-1 etc.) and the association of the labeled antibody with the cell can be measured by a suitable technique (e.g., immunohistochemistry, flow cytometry etc.).

A preferred method for altering an antigen on a donor cell to inhibit an immune response against the cell is to contact the cell with a molecule which binds to the antigen on the cell surface. It is preferred that the cell be contacted with the molecule which binds to the antigen prior to administering the cell to a recipient (i.e., the cell is contacted with the molecule *in vitro*). For example, the cell can be incubated with the molecule which binds the antigen under conditions which allow binding of the molecule to the antigen and then any unbound molecule can be removed. Following administration of the modified cell to a recipient, the molecule remains bound to the antigen on the cell for a sufficient time to interfere with immunological recognition by host cells and induce non-responsiveness in the recipient.

Preferably, the molecule for binding to an antigen on a donor cell is an antibody, or fragment or derivative thereof which retains the ability to bind to the antigen. For use in therapeutic applications, it is necessary that the antibody which binds the antigen to be altered be unable to fix complement, thus preventing donor cell lysis. Antibody complement fixation can be prevented by deletion of an Fc portion of an antibody, by using an antibody isotype which is not capable of fixing complement, or by using a complement fixing antibody in conjunction with a drug which inhibits complement fixation. Alternatively, amino acid residues within the Fc region which are necessary for activating complement (*see e.g.,* Tan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:162-166; Duncan and Winter (1988) *Nature* 332: 738-740) can be mutated to reduce or eliminate the complement-activating ability of an intact antibody. Likewise, amino acids residues within the Fc region which are necessary for binding of the Fc region to Fc receptors (*see e.g.,* Canfield, S.M. and S.L. Morrison (1991) *J. Exp. Med.* 173:1483-1491; and Lund, J. et al. (1991) *J. Immunol.* 147:2657-2662) can also be mutated to reduce or eliminate Fc receptor binding if an intact antibody is to be used.

A preferred antibody fragment for altering an antigen is an $F(ab')_2$ fragment. Antibodies can be fragmented using conventional techniques. For example, the Fc portion of an antibody can be removed by treating an intact antibody with pepsin, thereby generating an $F(ab')_2$ fragment. In a standard procedure for generating $F(ab')_2$ fragments, intact antibodies are incubated with immobilized pepsin and the digested antibody mixture is applied to an immobilized protein A column. The free Fc portion binds to the column while the $F(ab')_2$ fragments passes through the column. The $F(ab')_2$ fragments can be further purified by HPLC or FPLC. $F(ab')_2$ fragments can be treated to reduce disulfide bridges to produce Fab' fragments.

An antibody, or fragment or derivative thereof, to be used to alter an antigen can be derived from polyclonal antisera containing antibodies reactive with a number of epitopes on

an antigen. Preferably, the antibody is a monoclonal antibody directed against the antigen. Polyclonal and monoclonal antibodies can be prepared by standard techniques known in the art. For example, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with the antigen or with a cell which expresses the antigen (e.g., on the cell surface) to elicit an antibody response against the antigen in the mammal. Alternatively, tissue or a whole organ which expresses the antigen can be used to elicit antibodies. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with the antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein ((1975) *Nature* 256:495-497) as well as other techniques such as the human B-cell hybridoma technique (Kozbar et al., (1983) *Immunol. Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. (1985) *Monoclonal Antibodies in Cancer Therapy*, Allen R. Bliss, Inc., pages 77-96) can be used. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the antigen and monoclonal antibodies isolated.

Another method of generating specific antibodies, or antibody fragments, reactive against the antigen is to screen expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with the antigen (or a portion thereof). For example, complete Fab fragments, V_H regions, F_V regions and single chain antibodies can be expressed in bacteria using phage expression libraries. *See e.g.,* Ward et al., (1989) *Nature* 341:544-546; Huse et al., (1989) *Science* 246:1275-1281; and McCafferty et al. (1990) *Nature* 348:552-554. Alternatively, a SCID-hu mouse can be used to produce antibodies, or fragments thereof (available from Genpharm). Antibodies of the appropriate binding specificity which are made by these techniques can be used to alter an antigen on a donor cell.

An antibody, or fragment thereof, produced in a non-human subject can be recognized to varying degrees as foreign when the antibody is administered to a human subject (e.g., when a donor cell with an antibody bound thereto is administered to a human subject) and an immune response against the antibody may be generated in the subject. One approach for minimizing or eliminating this problem is to produce chimeric or humanized antibody derivatives, i.e., antibody molecules comprising portions which are derived from non-human antibodies and portions which are derived from human antibodies. Chimeric antibody molecules can include, for example, an antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. A variety of approaches for making chimeric antibodies have been described. *See e.g.,* Morrison et al., *Proc. Natl. Acad. Sci.*

U.S.A. 81, 6851 (1985); Takeda et al., *Nature* 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B. For use in therapeutic applications, it is preferred that an antibody used to alter a donor cell antigen not contain an Fc portion. Thus, a humanized $F(ab')_2$ fragment in which parts of the variable region of the antibody, especially the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin is a preferred antibody derivative. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (e.g., Teng et al., *Proc. Natl. Acad. Sci. U.S.A.*, 80, 7308-7312 (1983); Kozbor et al., *Immunology Today*, 4, 7279 (1983); Olsson et al., *Meth. Enzymol.*, 92, 3-16 (1982)), and are preferably made according to the teachings of PCT Publication WO92/06193 or EP 0239400. Humanized antibodies can be commercially produced by, for example, Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.

Each of the cell surface antigens to be altered, e.g., MHC class I antigens, MHC class II antigens, LFA-3 and ICAM-1 are well-characterized molecules and antibodies to these antigens are commercially available. For example, an antibody directed against human MHC class I antigens (i.e., an anti-HLA class I antibody),W6/32, is available from the American Type Culture Collection (ATCC HB 95). This antibody was raised against human tonsillar lymphocyte membranes and binds to HLA-A, HLA-B and HLA-C (Barnstable, C.J. et al. (1978) *Cell* 14:9-20). Another anti-MHC class I antibody which can be used is PT85 (see Davis, W.C. et al. (1984) *Hybridoma Technology in Agricultural and Veterinary Research.* N.J. Stern and H.R. Gamble, eds., Rownman and Allenheld Publishers, Totowa, NJ, p121; commercially available from Veterinary Medicine Research Development, Pullman, WA). This antibody was raised against swine leukocyte antigens (SLA) and binds to class I antigens from several different species (e.g., pig, human, mouse, goat). An anti-ICAM-1 antibody can be obtained from AMAC, Inc., Maine. Hybridoma cells producing anti-LFA-3 can be obtained from the American Type Culture Collection, Rockville, Maryland.

A suitable antibody, or fragment or derivative thereof, for use in the invention can be identified based upon its ability to inhibit the immunological rejection of allogeneic or xenogeneic cells. Briefly, the antibody (or antibody fragment) is incubated for a short period of time (e.g., 30 minutes at room temperature) with cells or tissue to be transplanted and any unbound antibody is washed away. The cells or tissue are then transplanted into a recipient animal. The ability of the antibody pretreatment to inhibit or prevent rejection of the transplanted cells or tissue is then determined by monitoring for rejection of the cells or tissue compared to untreated controls.

It is preferred that an antibody, or fragment or derivative thereof, which is used to alter an antigen have an affinity for binding to the antigen of at least 10^{-7} M. The affinity of an antibody or other molecule for binding to an antigen can be determined by conventional

techniques (see Masan, D.W. and Williams, A.F. (1980) *Biochem. J.* 187:1-10). Briefly, the antibody to be tested is labeled with ^{125}I and incubated with cells expressing the antigen at increasing concentrations until equilibrium is reached. Data are plotted graphically as [bound antibody]/[free antibody] versus [bound antibody] and the slope of the line is equal to the kD (Scatchard analysis).

Other molecules which bind to an antigen on a donor cell and produce a functionally similar result as antibodies, or fragments or derivatives thereof, (e.g., other molecules which interfere with the interaction of the antigen with a hematopoietic cell and induce immunological nonresponsiveness) can be used to alter the antigen on the donor cell. One such molecule is a soluble form of a ligand for an antigen (e.g., a receptor) on the donor cell which could be used to alter the antigen on the donor cell. For example, a soluble form of CD2 (i.e., comprising the extracellular domain of CD2 without the transmembrane or cytoplasmic domain) can be used to alter LFA-3 on the donor cell by binding to LFA-3 on donor cells in a manner analogous to an antibody. Alternatively, a soluble form of LFA-1 can be used to alter ICAM-1 on the donor cell. A soluble form of a ligand can be made by standard recombinant DNA procedures, using a recombinant expression vector containing DNA encoding the ligand encompassing an extracellular domain (i.e., lacking DNA encoding the transmembrane and cytoplasmic domains). The recombinant expression vector encoding the extracellular domain of the ligand can be introduced into host cells to produce a soluble ligand, which can then be isolated. Soluble ligands of use have a binding affinity for the receptor on the donor cell sufficient to remain bound to the receptor to interfere with immunological recognition and induce non-responsiveness when the cell is administered to a recipient (e.g., preferably, the affinity for binding of the soluble ligand to the receptor is at least about 10^{-7} M). Additionally, the soluble ligand can be in the form of a fusion protein comprising the receptor binding portion of the ligand fused to another protein or portion of a protein. For example, an immunoglobulin fusion protein which includes an extracellular domain, or functional portion of CD2 or LFA-1 linked to an immunoglobulin heavy chain constant region (e.g., the hinge, CH2 and CH3 regions of a human immunoglobulin such as IgG1) can be used. Immunoglobulin fusion proteins can be prepared, for example, according to the teachings of Capon, D.J. et al. (1989) *Nature* 337:525-531 and U.S. Patent No. 5,116,964 to Capon and Lasky.

Another type of molecule which can be used to alter an MHC antigen (e.g., and MHC class I antigen) is a peptide which binds to the MHC antigen and interferes with the interaction of the MHC antigen with a T lymphocyte, NK cell, or LAK cell. In one embodiment, the soluble peptide mimics a region of the T cell receptor which contacts the MHC antigen. This peptide can be used to interfere with the interaction of the intact T cell receptor (on a T lymphocyte) with the MHC antigen. Such a peptide binds to a region of the MHC molecule which is specifically recognized by a portion of the T cell receptor (e.g., the alpha-1 or alpha-2 domain of an MHC class I antigen), thereby altering the MHC class I

antigen and inhibiting recognition of the antigen by the T cell receptor. In another embodiment, the soluble peptide mimics a region of a T cell surface molecule which contacts the MHC antigen, such as a region of the CD8 molecule which contacts an MHC class I antigen or a region of a CD4 molecule which contacts an MHC class II antigen. For example, a peptide which binds to a region of the alpha-3 loop of an MHC class I antigen can be used to inhibit binding to CD8 to the antigen, thereby inhibiting recognition of the antigen by T cells. T cell receptor-derived peptides have been used to inhibit MHC class I-restricted immune responses (see e.g., Clayberger, C. et al. (1993) *Transplant Proc.* 25:477-478) and prolong allogeneic skin graft survival *in vivo* when injected subcutaneously into the recipient (see e.g., Goss, J.A. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:9872-9876).

An antigen on a donor cell further can be altered by using two or more molecules which bind to the same or different antigen. For example, two different antibodies with specificity for two different epitopes on the same antigen can be used (e.g., two different anti-MHC class I antibodies can be used in combination). Alternatively, two different types of molecules which bind to the same antigen can be used (e.g., an anti-MHC class I antibody and an MHC class I-binding peptide). A preferred combination of anti-MHC class I antibodies which can be used with human cells is the W6/32 antibody and the PT85 antibody or $F(ab')_2$ fragments thereof. When the donor cell to be administered to a subject bears more than one hematopoietic cell-interactive antigen, two or more treatments can be used together. For example, two antibodies, each directed against a different antigen (eg., an anti-MHC class I antibody and an anti-ICAM-1 antibody) can be used in combination or two different types of molecules, each binding to a different antigen, can be used (e.g., an anti-ICAM-1 antibody and an MHC class I-binding peptide). Alternatively, polyclonal antisera generated against the entire donor cell or tissue containing donor cells can be used, following removal of the Fc region, to alter multiple cell surface antigens of the donor cells.

The ability of two different monoclonal antibodies which bind to the same antigen to bind to different epitopes on the antigen can be determined using a competition binding assay. Briefly, one monoclonal antibody is labeled and used to stain cells which express the antigen. The ability of the unlabeled second monoclonal antibody to inhibit the binding of the first labeled monoclonal antibody to the antigen on the cells is then assessed. If the second monoclonal antibody binds to a different epitope on the antigen than does the first antibody, the second antibody will be unable to competitively inhibit the binding of the first antibody to the antigen.

A preferred method for altering at least two different epitopes on an antigen on a donor cell to inhibit an immune response against the cell is to contact the cell with at least two different molecules which bind to the epitopes. It is preferred that the cell be contacted with at least two different molecules which bind to the different epitopes prior to administering the cell to a recipient (i.e., the cell is contacted with the molecule *in vitro*). For example, the cell can be incubated with the molecules which bind to the epitopes under

conditions which allow binding of the molecules to the epitopes and then any unbound molecules can be removed. Following administration of the donor cell to a recipient, the molecules remain bound to the epitopes on the surface antigen for a sufficient time to interfere with immunological recognition by host cells and induce non-responsiveness in the recipient.

Alternative to binding a molecule (e.g., an antibody) to an antigen on a donor cell to inhibit immunological rejection of the cell, the antigen on the donor cell can be altered by other means. For example, the antigen can be directly altered (e.g., mutated) such that it can no longer interact normally with an immune cell, e.g., a T lymphocyte), an NK cell, or an LAK cell, in an allogeneic or xenogeneic recipient and induces immunological non-responsiveness to the donor cell in the recipient. For example, a mutated form of a class I MHC antigen or adhesion molecule (e.g., LFA-3 or ICAM-1) which does not contribute to T cell activation but rather delivers an inappropriate or insufficient signal to a T cell upon binding to a receptor on the T cell can be created by mutagenesis and selection. A nucleic acid encoding the mutated form of the antigen can then be inserted into the genome of a non-human animal, either as a transgene or by homologous recombination (to replace the endogenous gene encoding the wild-type antigen). Cells from the non-human animal which express the mutated form of the antigen can then be used as donor cells for transplantation into an allogeneic or xenogeneic recipient.

Alternatively, an antigen on the donor cell can be altered by downmodulating or altering its level of expression on the surface of the donor cell such that the interaction between the antigen and a recipient immune cell is modified. By decreasing the level of surface expression of one or more antigens on the donor cell, the avidity of the interaction between the donor cell and the immune cell e.g., T lymphocyte, NK cell, LAK cell, is reduced. The level of surface expression of an antigen on the donor cell can be down-modulated by inhibiting the transcription, translation or transport of the antigen to the cell surface. Agents which decrease surface expression of the antigen can be contacted with the donor cell. For example, a number of oncogenic viruses have been demonstrated to decrease MHC class I expression in infected cells (*see e.g.,* Travers et al. (1980) *Int'l. Symp. on Aging in Cancer,* 175180; Rees et al. (1988) *Br. J. Cancer*, 57:374-377). In addition, it has been found that this effect on MHC class I expression can be achieved using fragments of viral genomes, in addition to intact virus. For example, transfection of cultured kidney cells with fragments of adenovirus causes elimination of surface MHC class I antigenic expression (Whoshi et al. (1988) *J. Exp. Med.* 168:2153-2164). For purposes of decreasing MHC class I expression on the surfaces of donor cells, viral fragments which are non-infectious are preferable to whole viruses.

Alternatively, the level of an antigen on the donor cell surface can be altered by capping the antigen. Capping is a term referring to the use of antibodies to cause aggregation and inactivation of surface antigens. To induce capping, a tissue is contacted with a first

antibody specific for an antigen to be altered, to allow formation of antigen-antibody immune complexes. Subsequently, the tissue is contacted with a second antibody which forms immune complexes with the first antibody. As a result of treatment with the second antibody, the first antibody is aggregated to form a cap at a single location on the cell surface. The technique of capping is well known and has been described, e.g., in Taylor et al. (1971), *Nat. New Biol.* 233:225-227; and Santiso et al. (1986), *Blood*, 67:343-349. To alter MHC class I antigens, donor cells are incubated with a first antibody (e.g., W6/32 antibody, PT85 antibody) reactive with MHC class I molecules, followed by incubation with a second antibody reactive with the donor species, e.g., goat anti-mouse antibody, to result in aggregation.

In yet another embodiment, cells which are administered to a subject according to the methods of the invention are genetically modified to express a gene product. The genetically modified cells can be transplanted into a recipient subject to deliver the gene product to the subject. Cells can be genetically modified to express a gene product by introducing nucleic acid encoding the gene product into the cell. For example, a cell can be infected with a recombinant virus (e.g., retrovirus, adenovirus) which contains the nucleic acid of interest. A non-human cell which is genetically modified to express a human gene product can be used to deliver the human gene product to a human subject by altering at least one antigen on the surface of the non-human cell and transplanting the cell into the recipient subject.

A cell can modified to express a gene product by introducing genetic material, such as a nucleic acid molecule (e.g., RNA or, more preferably, DNA) into the cell. The nucleic acid molecule introduced into the cell encodes a gene product to be expressed by the cell. The term "gene product" as used herein is intended to include proteins, peptides and functional RNA molecules. Generally, the gene product encoded by the nucleic acid molecule is the desired gene product to be supplied to a subject. Alternatively, the encoded gene product is one which induces the expression of the desired gene product by the cell (e.g., the introduced genetic material encodes a transcription factor which induces the transcription of the gene product to be supplied to the subject).

A nucleic acid molecule introduced into a cell is in a form suitable for expression in the cell of the gene product encoded by the nucleic acid. Accordingly, the nucleic acid molecule includes coding and regulatory sequences required for transcription of a gene (or portion thereof) and, when the gene product is a protein or peptide, translation of the gene product encoded by the gene. Regulatory sequences which can be included in the nucleic acid molecule include promoters, enhancers and polyadenylation signals, as well as sequences necessary for transport of an encoded protein or peptide, for example N-terminal signal sequences for transport of proteins or peptides to the surface of the cell or for secretion.

Nucleotide sequences which regulate expression of a gene product (e.g., promoter and enhancer sequences) are selected based upon the type of cell in which the gene product is to be expressed and the desired level of expression of the gene product. For example, a

promoter known to confer cell-type specific expression of a gene linked to the promoter can be used. A promoter specific for myoblast gene expression can be linked to a gene of interest to confer muscle-specific expression of that gene product. Muscle-specific regulatory elements which are known in the art include upstream regions from the dystrophin gene (Klamut et al. (1989) *Mol. Cell. Biol.* 9:2396), the creatine kinase gene (Buskin and Hauschka (1989) *Mol. Cell Biol.* 9:2627) and the troponin gene (Mar and Ordahl (1988) *Proc. Natl. Acad. Sci. USA.* 85:6404). Regulatory elements specific for other cell types are known in the art (e.g., the albumin enhancer for liver-specific expression; insulin regulatory elements for pancreatic islet cell-specific expression; various neural cell-specific regulatory elements, including neural dystrophin, neural enolase and A4 amyloid promoters). Alternatively, a regulatory element which can direct constitutive expression of a gene in a variety of different cell types, such as a viral regulatory element, can be used. Examples of viral promoters commonly used to drive gene expression include those derived from polyoma virus, Adenovirus 2, cytomegalovirus and Simian Virus 40, and retroviral LTRs. Alternatively, a regulatory element which provides inducible expression of a gene linked thereto can be used. The use of an inducible regulatory element (e.g., an inducible promoter) allows for modulation of the production of the gene product in the cell. Examples of potentially useful inducible regulatory systems for use in eukaryotic cells include hormone-regulated elements (e.g., see Mader, S. and White, J.H. (1993) *Proc. Natl. Acad. Sci. USA* 90:5603-5607), synthetic ligand-regulated elements (see, e.g. Spencer, D.M. et al. (1993) *Science* 262:1019-1024) and ionizing radiation-regulated elements (e.g., see Manome, Y. et al. (1993) *Biochemistry* 32:10607-10613; Datta, R. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10149-10153). Additional tissue-specific or inducible regulatory systems which may be developed can also be used in accordance with the invention.

There are a number of techniques known in the art for introducing genetic material into a cell that can be applied to modify a cell of the invention. In one embodiment, the nucleic acid is in the form of a naked nucleic acid molecule. In this situation, the nucleic acid molecule introduced into a cell to be modified consists only of the nucleic acid encoding the gene product and the necessary regulatory elements. Alternatively, the nucleic acid encoding the gene product (including the necessary regulatory elements) is contained within a plasmid vector. Examples of plasmid expression vectors include CDM8 (Seed, B., *Nature* 329:840 (1987)) and pMT2PC (Kaufman, et al., *EMBO J.* 6:187-195 (1987)). In another embodiment, the nucleic acid molecule to be introduced into a cell is contained within a viral vector. In this situation, the nucleic acid encoding the gene product is inserted into the viral genome (or a partial viral genome). The regulatory elements directing the expression of the gene product can be included with the nucleic acid inserted into the viral genome (i.e.. linked to the gene inserted into the viral genome) or can be provided by the viral genome itself. Examples of methods which can be used to introduce naked nucleic acid into cells and viral-mediated transfer of nucleic acid into cells are described separately in the subsections below.

A. Introduction of Naked Nucleic Acid into Cells

1. *Transfection mediated by $CaPO_4$*: Naked DNA can be introduced into cells by forming a precipitate containing the DNA and calcium phosphate. For example, a HEPES-buffered saline solution can be mixed with a solution containing calcium chloride and DNA to form a precipitate and the precipitate is then incubated with cells. A glycerol or dimethyl sulfoxide shock step can be added to increase the amount of DNA taken up by certain cells. $CaPO_4$-mediated transfection can be used to stably (or transiently) transfect cells and is only applicable to *in vitro* modification of cells. Protocols for $CaPO_4$- mediated transfection can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Section 9.1 and in Molecular Cloning: A Laboratory Manual, 2nd Edition, Sambrook et al. Cold Spring Harbor Laboratory Press, (1989), Sections 16.32-16.40 or other standard laboratory manuals.

2. *Transfection mediated by DEAE-dextran*: Naked DNA can be introduced into cells by forming a mixture of the DNA and DEAE-dextran and incubating the mixture with the cells. A dimethylsulfoxide or chloroquine shock step can be added to increase the amount of DNA uptake. DEAE-dextran transfection is only applicable to *in vitro* modification of cells and can be used to introduce DNA transiently into cells but is not preferred for creating stably transfected cells. Thus, this method can be used for short term production of a gene product but is not a method of choice for long-term production of a gene product. Protocols for DEAE-dextran-mediated transfection can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Section 9.2 and in Molecular Cloning: A Laboratory Manual, 2nd Edition, Sambrook et al. Cold Spring Harbor Laboratory Press, (1989), Sections 16.41-16.46 or other standard laboratory manuals.

3. *Electroporation*: Naked DNA can also be introduced into cells by incubating the cells and the DNA together in an appropriate buffer and subjecting the cells to a high-voltage electric pulse. The efficiency with which DNA is introduced into cells by electroporation is influenced by the strength of the applied field, the length of the electric pulse, the temperature, the conformation and concentration of the DNA and the ionic composition of the media. Electroporation can be used to stably (or transiently) transfect a wide variety of cell types and is only applicable to *in vitro* modification of cells. Protocols for electroporating cells can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Section 9.3 and in Molecular Cloning: A Laboratory Manual, 2nd Edition, Sambrook et al. Cold Spring Harbor Laboratory Press, (1989), Sections 16.54-16.55 or other standard laboratory manuals.

4. *Liposome-mediated transfection ("lipofection")*: Naked DNA can be introduced into cells by mixing the DNA with a liposome suspension containing cationic lipids. The DNA/liposome complex is then incubated with cells. Liposome mediated transfection can be used to stably (or transiently) transfect cells in culture *in vitro*. Protocols can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Section 9.4 and other standard laboratory manuals. Additionally, gene delivery *in vivo* has been accomplished using liposomes. See for example Nicolau et al. (1987) *Meth. Enz.* 149:157-176; Wang and Huang (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855; Brigham et al. (1989) *Am. J. Med. Sci.* 298:278; and Gould-Fogerite et al. (1989) *Gene* 84:429-438.

5. *Direct Injection*: Naked DNA can be introduced into cells by directly injecting the DNA into the cells. For an *in vitro* culture of cells, DNA can be introduced by microinjection. Since each cell is microinjected individually, this approach is very labor intensive when modifying large numbers of cells. However, a situation wherein microinjection is a method of choice is in the production of transgenic animals (discussed in greater detail below). In this situation, the DNA is stably introduced into a fertilized oocyte which is then allowed to develop into an animal. The resultant animal contains cells carrying the DNA introduced into the oocyte. Direct injection has also been used to introduce naked DNA into cells *in vivo* (*see e.g.*, Acsadi et al. (1991) *Nature* 332: 815-818; Wolff et al. (1990) *Science* 247:1465-1468). A delivery apparatus (e.g., a "gene gun") for injecting DNA into cells *in vivo* can be used. Such an apparatus is commercially available (e.g., from BioRad).

6. *Receptor-Mediated DNA Uptake*: Naked DNA can also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C.H. (1988) *J. Biol. Chem.* 263:14621; Wilson et al. (1992) *J. Biol. Chem.* 267:963-967; and U.S. Patent No. 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. Receptors to which a DNA-ligand complex have targeted include the transferrin receptor and the asialoglycoprotein receptor. A DNA-ligand complex linked to adenovirus capsids which naturally disrupt endosomes, thereby releasing material into the cytoplasm can be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8850; Cristiano et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:2122-2126). Receptor-mediated DNA uptake can be used to introduce DNA into cells either *in vitro* or *in vivo* and, additionally, has the added feature that DNA can be selectively targeted to a particular cell type by use of a ligand which binds to a receptor selectively expressed on a target cell of interest.

Generally, when naked DNA is introduced into cells in culture (e.g., by one of the transfection techniques described above) only a small fraction of cells (about 1 out of 10^5)

typically integrate the transfected DNA into their genomes (i.e., the DNA is maintained in the cell episomally). Thus, in order to identify cells which have taken up exogenous DNA, it is advantageous to transfect nucleic acid encoding a selectable marker into the cell along with the nucleic acid(s) of interest. Preferred selectable markers include those which confer resistance to drugs such as G418, hygromycin and methotrexate. Selectable markers may be introduced on the same plasmid as the gene(s) of interest or may be introduced on a separate plasmid.

An alternative method for generating a cell that is modified to express a gene product involving introducing naked DNA into cells is to create a transgenic animal which contains cells modified to express the gene product of interest. A transgenic animal is an animal having cells that contain a transgene, wherein the transgene was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA molecule which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. Thus, a transgenic animal expressing a gene product of interest in one or more cell types within the animal can be created, for example, by introducing a nucleic acid encoding the gene product (typically linked to appropriate regulatory elements, such as a tissue-specific enhancer) into the male pronuclei of a fertilized oocyte, e.g., by microinjection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Methods for generating transgenic animals, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009 and Hogan, B. et al., (1986) A Laboratory Manual, Cold Spring Harbor, New York, Cold Spring Harbor Laboratory. A transgenic founder animal can be used to breed more animals carrying the transgene. Cells of the transgenic animal which express a gene product of interest can then be used to deliver the gene product to a subject in accordance with the invention.

Alternatively, an animal containing a gene which has been modified by homologous recombination can be constructed to express a gene product of interest. For example, an endogenous gene carried in the genome of the animal can be altered by homologous recombination (for instance, all or a portion of a gene could be replaced by the human homologue of the gene to "humanize" the gene product encoded by the gene) or an endogenous gene can be "knocked out" (i.e., inactivated by mutation). For example, an endogenous gene in a cell can be knocked out to prevent production of that gene product and then nucleic acid encoding a different (preferred) gene product is introduced into the cell. To create an animal with homologously recombined nucleic acid, a vector is prepared which contains the DNA which is to replace or interrupt the endogenous DNA flanked by DNA homologous to the endogenous DNA (see for example Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503). The vector is introduced into an embryonal stem cell line (e.g., by electroporation) and cells which have homologously recombined the DNA are selected (see

for example Li, E. et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see for example Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA. Cells of the animal containing the homologously recombined DNA which express a gene product of interest can then be used to deliver the gene product to a subject in accordance with the invention.

B. Viral-Mediated Gene Transfer

A preferred approach for introducing nucleic acid encoding a gene product into a cell is by use of a viral vector containing nucleic acid, e.g. a cDNA, encoding the gene product. Infection of cells with a viral vector has the advantage that a large proportion of cells receive the nucleic acid, which can obviate the need for selection of cells which have received the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid and viral vector systems can be used either *in vitro* or *in vivo*.

1. *Retroviruses*: Defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76:271). A recombinant retrovirus can be constructed having a nucleic acid encoding a gene product of interest inserted into the retroviral genome. Additionally, portions of the retroviral genome can be removed to render the retrovirus replication defective. The replication defective retrovirus is then packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include ψCrip, ψCre, ψ2 and ψAm. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, *in vitro* and/or *in vivo* (see for example Eglitis, et al. (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury et al. (1991) *Science*

254:1802-1805; van Beusechem et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay et al. (1992) *Human Gene Therapy* 3:641-647; Dai et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu et al. (1993) *J. Immunol.* 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Retroviral vectors require target cell division in order for the retroviral genome (and foreign nucleic acid inserted into it) to be integrated into the host genome to stably introduce nucleic acid into the cell. Thus, it may be necessary to stimulate replication of the target cell.

2. *Adenoviruses*: The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner et al. (1988) *BioTechniques* 6:616; Rosenfeld et al. (1991) *Science* 252:431-434; and Rosenfeld et al. (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types, including airway epithelium (Rosenfeld et al. (1992) cited *supra*), endothelial cells (Lemarchand et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6482-6486), hepatocytes (Herz and Gerard (1993) *Proc. Natl. Acad. Sci. USA* 90:2812-2816) and muscle cells (Quantin et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:2581-2584). Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner et al. cited *supra*; Haj-Ahmand and Graham (1986) *J. Virol.* 57:267). Most replication-defective adenoviral vectors currently in use are deleted for all or parts of the viral E1 and E3 genes but retain as much as 80% of the adenoviral genetic material.

3. *Adeno-Associated Viruses*: Adeno-associated virus (AAV) is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. *Curr. Topics in Micro. and Immunol.* (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski et al. (1989) *J. Virol.* 63:3822-3828; and McLaughlin et al. (1989) *J. Virol.* 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as

that described in Tratschin et al. (1985) *Mol. Cell. Biol.* 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470; Tratschin et al. (1985) *Mol. Cell. Biol.* 4:2072-2081; Wondisford et al. (1988) *Mol. Endocrinol.* 2:32-39; Tratschin et al. (1984) *J. Virol.* 51:611-619; and Flotte et al. (1993) *J. Biol. Chem.* 268:3781-3790).

The efficacy of a particular expression vector system and method of introducing nucleic acid into a cell can be assessed by standard approaches routinely used in the art. For example, DNA introduced into a cell can be detected by a filter hybridization technique (e.g., Southern blotting) and RNA produced by transcription of introduced DNA can be detected, for example, by Northern blotting, RNase protection or reverse transcriptase-polymerase chain reaction (RT-PCR). The gene product can be detected by an appropriate assay, for example by immunological detection of a produced protein, such as with a specific antibody, or by a functional assay to detect a functional activity of the gene product, such as an enzymatic assay. If the gene product of interest to be expressed by a cell is not readily assayable, an expression system can first be optimized using a reporter gene linked to the regulatory elements and vector to be used. The reporter gene encodes a gene product which is easily detectable and, thus, can be used to evaluate the efficacy of the system. Standard reporter genes used in the art include genes encoding β-galactosidase, chloramphenicol acetyl transferase, luciferase and human growth hormone.

When the method used to introduce nucleic acid into a population of cells results in modification of a large proportion of the cells and efficient expression of the gene product by the cells (e.g., as is often the case when using a viral expression vector), the modified population of cells may be used without further isolation or subcloning of individual cells within the population. That is, there may be sufficient production of the gene product by the population of cells such that no further cell isolation is needed. Alternatively, it may be desirable to grow a homogenous population of identically modified cells from a single modified cell to isolate cells which efficiently express the gene product. Such a population of uniform cells can be prepared by isolating a single modified cell by limiting dilution cloning followed by expanding the single cell in culture into a clonal population of cells by standard techniques.

C. Other Methods for Modifying a Cell to Express a Gene Product

Alternative to introducing a nucleic acid molecule into a cell to modify the cell to express a gene product, a cell can be modified by inducing or increasing the level of expression of the gene product by a cell. For example, a cell may be capable of expressing a particular gene product but fails to do so without additional treatment of the cell. Similarly, the cell may express insufficient amounts of the gene product for the desired purpose. Thus, an agent which stimulates expression of a gene product can be used to induce or increase

expression of a gene product by the cell. For example, cells can be contacted with an agent *in vitro* in a culture medium. The agent which stimulates expression of a gene product may function, for instance, by increasing transcription of the gene encoding the product, by increasing the rate of translation or stability (e.g., a post transcriptional modification such as a poly A tail) of an mRNA encoding the product or by increasing stability, transport or localization of the gene product. Examples of agents which can be used to induce expression of a gene product include cytokines and growth factors.

Another type of agent which can be used to induce or increase expression of a gene product by a cell is a transcription factor which upregulates transcription of the gene encoding the product. A transcription factor which upregulates the expression of a gene encoding a gene product of interest can be provided to a cell, for example, by introducing into the cell a nucleic acid molecule encoding the transcription factor. Thus, this approach represents an alternative type of nucleic acid molecule which can be introduced into the cell (for example by one of the previously discussed methods). In this case, the introduced nucleic acid does not directly encode the gene product of interest but rather causes production of the gene product by the cell indirectly by inducing expression of the gene product.

In yet another method, a cell is modified to express a gene product by coupling the gene product to the cell, preferably to the surface of the cell. For example, a protein can be obtained by purifying the cell from a biological source or expressing the protein recombinantly using standard recombinant DNA technology. The isolated protein can then be coupled to the cell. The terms "coupled" or "coupling" refer to a chemical, enzymatic or other means (e.g., by binding to an antibody on the surface of the cell or genetic engineering of linkages) by which a gene product can be linked to a cell such that the gene product is in a form suitable for delivering the gene product to a subject. For example, a protein can be chemically crosslinked to a cell surface using commercially available crosslinking reagents (Pierce, Rockford IL). Other approaches to coupling a gene product to a cell include the use of a bispecific antibody which binds both the gene product and a cell-surface molecule on the cell or modification of the gene product to include a lipophilic tail (e.g., by inositol phosphate linkage) which can insert into a cell membrane.

In yet another embodiment, a recipient subject into which altered cells of the invention are transplanted is also treated with a T cell inhibitory agent to further inhibit rejection of the transplanted cells. The T cell inhibitory agent inhibits T cell activity. For example, the T cell inhibitory agent can be an immunosuppressive drug. A preferred immunosuppressive drug is cyclosporin A. Other immunosuppressive drugs which can be used include FK506 and RS-61443. Such immunosuppressive drugs can be used in conjunction with a steroid (e.g., glucocorticoids such as prednisone, methylprednisolone and dexamethasone) or chemotherapeutic agents (e.g., azathioprine and cyclophosphamide), or both. Alternatively, the T cell inhibitory agent can be one or more antibodies which deplete

T cell activity, such as antibodies directed against T cell surface molecules (e.g., anti-CD2, anti-CD3, anti-CD4 and/or anti-CD8 antibodies).

II. Methods of the Invention

Another aspect of the invention pertains to methods for reducing the immunogenicity of a cell for transplantation wherein the cell has at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject. These methods include contacting the cell with at least one molecule which binds to the antigen on the cell surface such that, when the cell is transplanted into a recipient subject, rejection of the cell is inhibited. The term "contacting" is intended to encompass either incubating the cell with the molecule which binds to the cell surface antigen *in vitro* or administering the molecule which binds to the cell surface antigen to a subject (e.g., a transplant recipient). In a preferred embodiment, it is the NK cell-mediated rejection or the LAK cell-mediated rejection of the cell which is inhibited. As used herein, the phrase "NK cell-mediated rejection" refers to an immune response which can lead to or does lead to rejection of a cell *in vivo*, or lysis of a cell *in vitro* and in which natural killer cells play either a direct or an indirect role. For example, NK cells can kill target cells by at least two mechanisms: antibody dependent cellular cytotoxicity (ADCC) or antibody independent cellular cytotoxicity. NK cells are characterized by the expression of the low affinity receptor for IgG, Fc-gamma-RIII (CD16) and neural cell adhesion molecule (NCAM, CD56). Binding of the NK cell CD16 to the Fc region of an IgG-coated cell results in lysis of the target (ADCC). Cytotoxicity by the antibody independent mechanism does not appear to require CD16 (Trinchieri, G. (1994) *J. Exp. Med.* 180:417-421). As used herein, the phrase "LAK cell-mediated rejection" refers to an immune response which can lead to or does lead to rejection of a cell *in vivo*, or lysis of a cell *in vitro* and in which lymphokine activated killer cells play either a direct or an indirect role. LAK cells and NK cells share many of the same cell surface markers. LAK cells are positive for the cell surface markers CD56, CD16, and CD25. LAK cells can be distinguished from NK cells in that they lyse certain cells types, e.g., Daudi cells (*see* Figure 9) while NK cells do not. An inhibition of rejection of cells of the present invention refers to prolongation of the cells' survival or prevention of rejection of the cells. Cells which can be used in these methods and methods of altering the cells are described in Section I above.

After a cell is modified or altered as described above, the cell can be administered to a recipient. Accordingly, another aspect of the invention pertains to methods for transplanting a cell into a recipient subject such that rejection of the cell by the recipient subject is inhibited. As used herein, the term "subject" is intended to include living organisms in which an immune response is elicited against allogeneic or xenogeneic cells, e.g., mammals, preferably humans. Other examples of subjects include monkeys, pigs, dogs, cats, mice, rats, and transgenic species thereof. A "recipient subject" is a subject into which cells have been transplanted or are to be transplanted. A recipient subject can be allogeneic to the

transplanted cells (i.e., of the same species) or can be xenogeneic to the transplanted cells (i.e., of a different species). The methods involve administering to the subject a cell having at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject. Prior to transplantation, the cell is modified or altered as described above such that rejection of the cell is inhibited. In preferred embodiments, the mechanisms of rejection which are inhibited are T cell-mediated, NK cell-mediated, and/or LAK cell-mediated rejection of the cell.

The cell is administered to the subject in an amount and by a route which suitable for the desired therapeutic result. The cell used in these methods can be within a tissue or organ. Accordingly, in these embodiments, the tissue or organ is transplanted into the recipient by conventional techniques for transplantation. Acceptance of transplanted cells, tissues or organs can be determined morphologically (e.g., with skin grafts by examining the transplanted tissue or by biopsy) or by assessment of the functional activity of the graft. For example, acceptance of pancreatic islet cells can be determined by measuring insulin production, acceptance of liver cells can be determined by assessing albumin production and acceptance of neural cells can be determined by assessing neural cell function. To determine whether, for example, the mechanism of rejection that is inhibited is NK cell-mediated rejection, NK cells can be isolated from the recipient subject's circulation or from a site in or near the graft (e.g., from a lymph node draining the graft area), or from a tissue section of the graft. The NK cells can then be cultured and their response to cells of the same type as those that were transplanted into the recipient subject can be measured. If the NK cells appear nonresponsive to the transplant cells relative to control NK cells or NK cells cultured under the same conditions, then NK cell-mediated rejection is most likely inhibited. To determine whether, for example, the mechanism of rejection that is inhibited is LAK cell-mediated rejection, the above experiments can be repeated wherein LAK cells are substituted for NK cells.

The methods of present invention can include additional *in vitro* treatment of the cells prior to transplantation and/or additional *in vivo* treatment of the recipient following transplantation to further inhibit immunological rejection of transplanted cells. For example, an antigen on a donor cell can be altered by using two or more molecules which bind to the same or different antigen as described in Section I above. In addition, a recipient subject can be treated prior to, during and/or following transplantation with an agent which inhibits T cell activity in the subject. The temporal relationship between administration of the cell and administration of the agent depends in part upon the nature of the agent used to inhibit T cell activity. Typically, the two compositions are administered contemporaneously, e.g. within several days of each other. Preferably, the cell and the agent are administered to the subject simultaneously or the agent is administered to the subject prior to administration of the cell.

As used herein, an agent which inhibits T cell activity is defined as an agent which results in removal (e.g., sequestration) or destruction of T cells within a subject or inhibits T

cell functions within the subject (i.e., T cells may still be present in the subject but are in a non-functional state, such that they are unable to proliferate or elicit or perform effector functions, e.g., cytokine production, cytotoxicity etc.). The term "T cell" encompasses mature peripheral blood T cells lymphocytes. The agent which inhibits T cell activity may also inhibit the activity or maturation of immature T cells (e.g., thymocytes).

A preferred agent for use in inhibiting T cell activity in a recipient subject is an immunosuppressive drug. The term "immunosuppressive drug" is intended to include pharmaceutical agents which inhibit or interfere with normal immune function. A preferred immunosuppressive drug is cyclosporin A. Other immunosuppressive drugs which can be used include FK506 and RS-61443. In one embodiment, the immunosuppressive drug is administered in conjunction with at least one other therapeutic agent. Additional therapeutic agents which can be administered include steroids (e.g., glucocorticoids such as prednisone, methyl prednisolone and dexamethasone) and chemotherapeutic agents (e.g., azathioprine and cyclosphosphamide). In another embodiment, an immunosuppressive drug is administered in conjunction with both a steroid and a chemotherapeutic agent. Suitable immunosuppressive drugs are commercially available (e.g., cyclosporin A is available from Sandoz, Corp., East Hanover, NJ).

An immunosuppressive drug is administered in a formulation which is compatible with the route of administration. Suitable routes of administration include intravenous injection (either as a single infusion, multiple infusions or as an intravenous drip over time), intraperitoneal injection, intramuscular injection and oral administration. For intravenous injection, the drug can be dissolved in a physiologically acceptable carrier or diluent (e.g., a buffered saline solution) which is sterile and allows for syringability. Dispersions of drugs can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Convenient routes of administration and carriers for immunosuppressive drugs are known in the art. For example, cyclosporin A can be administered intravenously in a saline solution, or orally, intraperitoneally or intramuscularly in olive oil or other suitable carrier or diluent.

An immunosuppressive drug is administered to a recipient subject at a dosage sufficient to achieve the desired therapeutic effect (e.g., inhibition of rejection of transplanted cells). Dosage ranges for immunosuppressive drugs, and other agents which can be coadministered therewith (e.g., steroids and chemotherapeutic agents), are known in the art (see e.g., Freed et al. *New Engl. J. Med.* (1992) 327:1549: Spencer et al. (1992) *New Engl. J. Med.* 327:1541; Widner et al. (1992) *New Engl. J. Med.* 327:1556; Lindvall et al. (1992) *Ann. Neurol.* 31:155; and Lindvall et al. (1992) *Arch. Neurol.* 46:615). A preferred dosage range for immunosuppressive drugs, suitable for treatment of humans, is about 1-30 mg/kg of body weight per day. A preferred dosage range for cyclosporin A is about 1-10 mg/kg of body weight per day, more preferably about 1-5 mg/kg of body weight per day. Dosages can be adjusted to maintain an optimal level of the immunosuppressive drug in the serum of the recipient subject. For example, dosages can be adjusted to maintain a preferred serum level

for cyclosporin A in a human subject of about 100-200 ng/ml. It is to be noted that dosage values may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted over time to provide the optimum therapeutic response according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

In one embodiment of the invention, an immunosuppressive drug is administered to a subject transiently for a sufficient time to induce tolerance to the transplanted cells in the subject. Transient administration of an immunosuppressive drug has been found to induce long-term graft-specific tolerance in a graft recipient (see Brunson et al. (1991) *Transplantation* 52:545; Hutchinson et al. (1981) *Transplantation* 32:210; Green et al. (1979) *Lancet* 2:123; Hall et al. (1985) *J. Exp. Med.* 162:1683). Administration of the drug to the subject can begin prior to transplantation of the cells into the subject. For example, initiation of drug administration can be a few days (e.g., one to three days) before transplantation. Alternatively, drug administration can begin the day of transplantation or a few days (generally not more than three days) after transplantation. Administration of the drug is continued for sufficient time to induce donor cell-specific tolerance in the recipient such that donor cells will continue to be accepted by the recipient when drug administration ceases. For example, the drug can be administered for as short as three days or as long as three months following transplantation. Typically, the drug is administered for at least one week but not more than one month following transplantation. Induction of tolerance to the transplanted cells in a subject is indicated by the continued acceptance of the transplanted cells after administration of the immunosuppressive drug has ceased. Acceptance of transplanted tissue can be determined morphologically (e.g., with skin grafts by examining the transplanted tissue or by biopsy) or by assessment of the functional activity of the graft. For example, acceptance of pancreatic islet cells can be determined by measuring insulin production, acceptance of liver cells can be determined by assessing liver function or acceptance of neural cells can be determined by assessing neural cell function.

Another type of agent which can be used to inhibit T cell activity in a subject is an antibody, or fragment or derivative thereof, which depletes or sequesters T cells in a recipient. Antibodies which are capable of depleting or sequestering T cells *in vivo* when administered to a subject are known in the art. Typically, these antibodies bind to an antigen on the surface of a T cell. Polyclonal antisera can be used, for example anti-lymphocyte serum. Alternatively, one or more monoclonal antibodies can be used. Preferred T cell-depleting antibodies include monoclonal antibodies which bind to CD2, CD3, CD4 or CD8 on the surface of T cells. Antibodies which bind to these antigens are known in the art and are available (e.g., from American Type Culture Collection). A preferred monoclonal antibody for binding to CD3 on human T cells is OKT3 (ATCC CRL 8001). The binding of

an antibody to surface antigens on a T cell can facilitate sequestration of T cells in a subject and/or destruction of T cells in a subject by endogenous mechanisms. Alternatively, a T cell-depleting antibody which binds to an antigen on a T cell surface can be conjugated to a toxin (e.g., ricin) or other cytotoxic molecule (e.g., a radioactive isotope) to facilitate destruction of T cells upon binding of the antibody to the T cells.

Another type of antibody which can be used to inhibit T cell activity in a recipient subject is an antibody which inhibits T cell proliferation. For example, an antibody directed against a T cell growth factor, such as IL-2, or a T cell growth factor receptor, such as the IL-2 receptor, can inhibit proliferation of T cells (*see e.g.,* DeSilva, D.R. et al. (1991) *J. Immunol.* 147:3261-3267). Accordingly, an anti-IL-2 or an anti-IL-2 receptor antibody can be administered to a recipient to inhibit rejection of a transplanted cell (*see e.g.* Wood et al. (1992) *Neuroscience* 49:410). Additionally, both an anti-IL-2 and an anti-IL-2 receptor antibody can be coadministered to inhibit T cell activity or can be administered with another antibody (e.g., which binds to a surface antigen on T cells).

An antibody which depletes, sequesters or inhibits T cells within a recipient can be administered at a dose and for an appropriate time to inhibit rejection of cells upon transplantation. Antibodies are preferably administered intravenously in a pharmaceutically acceptable carrier or diluent (e.g., a sterile saline solution). Antibody administration can begin prior to transplantation (e.g., one to five days prior to transplantation) and can continue on a daily basis after transplantation to achieve the desired effect (e.g., up to fourteen days after transplantation). A preferred dosage range for administration of an antibody to a human subject is about 0.1-0.3 mg/kg of body weight per day. Alternatively, a single high dose of antibody (e.g., a bolus at a dosage of about 10 mg/kg of body weight) can be administered to a human subject on the day of transplantation. The effectiveness of antibody treatment in depleting T cells from the peripheral blood can be determined by comparing T cell counts in blood samples taken from the subject before and after antibody treatment. Dosage regima can be adjusted over time to provide the optimum therapeutic response according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

III. Uses of the Method of the Invention

Cells having at least one surface antigen altered according to the invention can be administered to a subject (i.e., transplanted into the subject) for therapeutic purposes. A cell can be administered to a subject by any appropriate route which results in delivery of cell to a desired location in the subject. For example, cells can be administered intravenously, subcutaneously, intramuscularly, intracerebrally, subcapsularly (e.g., under the kidney capsule) or intraperitoneally. Cells can be administered in a physiologically compatible carrier, such as a buffered saline solution. When cells are within a tissue or organ, the tissue

or organ can be transplanted into a suitable location in the subject by conventional techniques to administer the cells to the subject.

The methods of the invention can be applied to any type of cell which is suitable for transplantation (i.e., any type of cell which can be isolated or obtained in a form that can be transplanted to another subject). The cells can be human cells or non-human cells. Preferred non-human cells are porcine cells. Preferred cell types for use in the method of the invention are cells which can provide a therapeutic function in a disease or disorder. Examples of such cells include muscle cells (e.g., myoblasts, myocytes, myotubes), liver cells, pancreatic islet cells, neural cells, e.g., mesencephalic cells, striatal cells, and cortical cells, and hematopoietic cells. For example, muscle cells can be transplanted into subjects suffering from a muscular dystrophy (e.g., Duchenne muscular dystrophy), pancreatic islet cells can be transplanted into a subject suffering from diabetes, neural cells can be transplanted into a subject suffering from Parkinson's disease, Huntington's disease, Alzheimer's disease, or epilepsy, liver cells can be transplanted into a subject with hepatic cell dysfunction (e.g. in hypercholesterolemia, hemophilia B or inherited emphysema), and hematopoietic cells can be transplanted into patients with hematopoietic or immunological dysfunction. Liver tissue can be obtained, for example, from brain dead donors or from non-human animals such as pigs. The cells can be dissociated by digestion with collagenase. Viable cells can be obtained and washed by centrifugation (at 700 x g), elution, and resuspension. At least one antigen on the surface of the liver cells (e.g., MHC class I antigen) is altered as described herein. Following alteration of the antigen(s), cells are administered through the portal vein to the liver of the recipient patient. In another embodiment, nerve cells obtained from a source (such as an abortus) are treated to alter a surface antigen and stereotaxically localized into the desired area of the brain, such as the corpus striatum or hippocampus. Dopaminergic or GABA-ergic neurons are used for the treatment of Parkinson's or Huntington's disease, respectively. In another embodiment, muscle cells can be obtained from a donor (e.g., by biopsy of a living related donor or from a brain dead donor) using a 14-16 gauge cutting trochar into a 1-2 inch skin incision. The fresh muscle plug can be lightly digested to a single cell suspension using collagenase, trypsin and dispase at 37°C. Floating debris is removed with a pipette and media washes and the viable cell pellet is counted after centrifugation at 1000 rpm for 10 minutes. The cell count is then used to calculate the amount of antibody fragments (or other suitable molecule, e.g. peptide) to be used to alter a surface antigen on the muscle cells. Muscle cells are injected intramuscularly into a recipient patient in need of an increased store of muscle, e.g., an elderly patient with severe muscle wasting, or injected into a muscle group of a patient afflicted with Becker's or Duchenne muscular dystrophy.

Recipient subjects are further treated with a T cell inhibitory agent according to the invention. Treatment can begin prior to, concurrent with or following transplantation of cells. The combination therapy taught by the invention provides a therapeutic regimen for transplantation of allogeneic or xenogeneic cells into a recipient subject which is more

effective than either alteration of donor cell surface antigens or treatment of the recipient with a T cell inhibitory agent alone.

This invention is further illustrated by the following Examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated by reference.

THE FOLLOWING MATERIALS AND METHODS WERE USED IN EXAMPLES I AND II:

Cells, culture media and reagents

Culture media consisted of RPMI supplemented with 10% human AB negative (or fetal calf) heat inactivated serum, 2mM L-glutamine, Penicillin (100U/ml), Streptomycin (100 mg/ml), and 30mM HEPES. Human and porcine PBLs were isolated from whole blood fractionated on Ficoll/Hypaque. PK15 cells are a transformed pig kidney cell line purchased from American Type Culture Collection (Accession No.: CCL 33).

Preparation of F(ab')2 fragments

$F(ab')_2$ fragments of antibodies W6/32 and PT85 were generated using immobilized pepsin, as follows. Purified antibody was added, at 20 mg/ml in pH 4.7 digestion buffer and digested for 4.0 hours. The crude digest was removed from the pepsin and immediately neutralized with pH 7.0 binding buffer. The antibody mixture was applied to an immobilized Protein A column and the elute was collected for the $F(ab')_2$ fragments. Dialysis against phosphate buffered saline for 24 h using 50,000 molecular weight cut-off tubing was then performed to rid the digest of contaminating Fc fragments. CHAPS buffer was added to the dialysis bag at a concentration of 10mM. The completeness of the digest and purification of the $F(ab')_2$ were monitored by silver staining of 15% SDS polyacrylamide gels. Final purification of the fragments was achieved by using a Superose 12 HPLC column. The completeness of Fc removal was demonstrated in an *in vitro* assay in which binding of the material to a target cell was followed with the addition of complement, and cytolysis of the pre-loaded target cells was measured by chromium release.

$F(ab')_2$ fragments were incubated with porcine cells described herein at a concentration of 1 μg of antibody per approximately 1 million cells for 30 min. at room temperature. After incubation, porcine cells were washed once with Hanks balanced salt solution containing 2% heat-inactivated fetal calf serum.

Cytolytic assays.

The cytolytic activity of freshly isolated human PBLs was assessed in a 4 hour ^{51}Cr release assay in which effector cells were tested against porcine PBLs. Targets were treated for 3 days with PHA (1mg/ml) in order to blast the cells and labeled with ^{51}Cr for 1 hour at

37°C. Targets were used at 5 x 10^3/well. Percent specific lysis was determined as the (Experimental cpm - spontaneous cpm/Maximum cpm-spontaneous cpm) x 100 = % cytotoxicity.

Mixed lymphocyte reaction

PK15 cells were grown on tissue culture flasks, trypsinized and replated at 1 x 10^4 cells/ml in DMEM with 10% FCS and added to 24 well flat bottom plates. Cells were allowed to adhere overnight at 37°C. The cells were then treated with mitomycin C (100 mg/ml) in serum free DMEM for 1 hour at 37°C. The cells were washed 3 times and prepared for masking. PT85 $IgG_2F(ab')_2$ was added to the appropriate wells at 10 µg/ml. After 2 hours at 4°C or 16 hours at 37°C all wells were washed 3 times in PBS. Human PBLs were added at 2 x 10^6 cells/well in a final volume of 2 ml. Plates were incubated for six days at 37°C, % CO_2 and then harvested for FACS analysis. Cell samples from each well were pulsed with ^{3}H-thymidine for 16 hours and then triplicate samples of 200 µl were harvested an a Packard Filtermate 196 cell harvester onto 96 well Unifilter plates. Incorporated radioactivity was measured on a Packard Top Count microplate scintillation counter.

Flow cytofluorimetric analysis

One x 10^5 cells were stained with the appropriate antibody followed by fluoresceinated goat anti-mouse IgG. Control samples were stained with the fluoresceinated reagent alone. All samples were then analyzed on a flow cytometer (FACScan, Becton Dickinson and Co), gated to remove nonviable cells.

EXAMPLE I: LYSIS OF PORCINE CELLS BY HUMAN CELLS IN THE ABSENCE OF PRIMING

NK cells are known to lyse target cells nonspecifically by a non-MHC restricted mechanism in the absence of priming whereas resting T or B lymphocytes should not mediate spontaneous killing (Moretta, L. et al. (1994) *Adv. Immunol.* 55:341-380). To determine whether freshly isolated human PBLs lyse porcine cells, a cytolytic assay was conducted as described in the Materials and Methods Section above. Freshly isolated human PBLs were able to lyse ^{51}Cr labeled porcine PBLs and hepatocytes. *See* Figures 1 and 2. In a control incubation, freshly isolated human PBLs were not able to lyse ^{51}Cr labeled allogeneic cells. The same cytolytic assays were performed in the presence of human serum and in the presence of fetal calf serum. The results of these assays (*see* Figures 1 and 2) indicated that lysis of porcine targets is not due to ADCC as a result of human antibodies that recognize pig cells since a similar level of lysis were seen when the assay was done in the presence of

human serum (presumably containing natural antibodies) and in the presence of fetal calf serum (containing no natural antibodies).

EXAMPLE II: EXPANSION OF NATURAL KILLER CELLS IN MIXED LYMPHOCYTE REACTION

Unprimed lysis of porcine cells by human PBLs shows a role for NK cells in killing of porcine cells. In order to address what role NK cells may play in masking, a test was performed to determine if NK cells could be detected in an *in vitro* assay wherein both antibody-treated and untreated porcine cells were cultured with human PBLs. In a mixed lymphocyte reaction (MLR; see Abbas, A.K. et al. (1994) Cellular and Molecular Immunology, 2nd ed. (W.B. Saunders Company, Philadelphia) pp. 341-343, for a general description of an MLR), porcine cell stimulation of human PBL proliferation was measured by incorporation of ^{3}H-thymidine (Figure 3). Treatment of the porcine cells with anti-class I monoclonal antibody, PT85, $F(ab')_2$ fragments resulted in reduced stimulation of human PBLs as measured in CPM (Figure 3).

Cells from the MLR were stained with monoclonal antibodies for cell surface markers and analyzed by FACS. Figure 4 shows the results of FACS staining of human cells isolated from the MLR. CD56 (NCAM) is a marker for NK cells. When human PBLs were stimulated with untreated porcine cells in the MLR, a remarkable increase in cells expressing CD56 was observed. However, when human PBLs were stimulated by treated porcine cells, a large increase in CD56 staining cells was not observed. *See* Figure 4. NK cells, like T cells, express the IL-2 receptor (CD25).

The results in Examples I and II above show a role for NK cells in the human anti-porcine response *in vitro*. Unprimed lysis of porcine cells can be detected in a ^{51}Cr release assay and allogeneic cells are not lysed. The human anti-porcine MLR shows an outgrowth of CD56 positive cells when the stimulator cells are untreated. This population of cells does not appear to grow out when the stimulators have been pretreated with the masking antibody.

THE FOLLOWING MATERIALS AND METHODS WERE USED IN EXAMPLES III-VII:

Preparation of peripheral blood lymphocytes

PBLs were isolated from human or pig whole blood by Ficoll-Hypaque gradient centrifugation. Coligan, J.E. et al. (1991) Current Protocols in Immunology Vol. 2, Coico, R. ed. (John Wiley and Sons, New York) ch. 7. Human blood was donated by healthy volunteers. Porcine blood was from Yorkshire and Hanford strains (Tufts School of

Veterinary Medicine) and from inbred minipigs (Massachusetts General Hospital) of the *aa or dd* haplotypes. Sachs, D.H. et al. (1976) *Transplantation* 22:559.

Cell lines, culture conditions and media

K562, JY and Daudi cells were obtained from the American Type Culture Collection. Cultured cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS) (Hyclone) and 100 U/ml penicillin/100 µg/ml streptomycin (BioWhittaker) or 50 µg/ml gentamycin (Gibco) in a 37°C humidified incubator with 5% CO_2. For cytotoxicity assays and mixed culture conditions, AIM-V (Gibco) media, which is serum free, was used. When media containing human serum was needed, RPMI-1640 (BioWhittaker) was supplemented with 10% AB pooled heat-inactivated human serum (PelFreeze), 2 mM glutamine (BioWhittaker), 100 U/ml penicillin/100 µg/ml streptomycin (BioWhittaker) and 10 mM HEPES (BioWhittaker). The media used in preparation of target cells was RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin/100 µg/ml streptomycin or 50 µg/ml gentamycin and 10 mM HEPES.

Cytotoxicity assays

Chromium release assays were performed basically as described (Coligan, J.E. et al. (1991) Current Protocols in Immunology Vol. 2, Coico, R. ed. (John Wiley and Sons, New York) ch. 7). Briefly, porcine PBLs used as targets were treated for 3 days with 5 µg/ml concanavalin A (Sigma). JY cells used as targets were harvested and resuspended in media. Target cells were labeled with ^{51}Cr. The cells were then washed 3 times before addition to the assay mix. Effector PBLs were added to a 96-well round-bottom microtiter plate ranging from 2.5 x 10^4/well to 5 x 10^5/ well. Targets were added at 2 to 5 x 10^3/well in a total of 200 µl. The plate was centrifuged for 4 minutes at 750 rpm to allow cell-cell contact. The plate was then incubated for 3 to 4 hours in a 37°C humidified incubator with 5% CO_2. One hundred microliters of supernatant was placed in a Luma plate (Packard). The plate was left to dry for 18 to 20 hours and then read in a TopCount scintillation counter (Packard). Percent specific lysis was determined by the following formula:

$$\% \text{ Specific Lysis} = \frac{100 \times (\text{experimental} - \text{spontaneous release})}{(\text{maximum} - \text{spontaneous release})}$$

For cold target inhibition assays (Colonna, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:1200), the effector to target ratio was 100 to 1. K562, Daudi or JY cells were added directly to the cytotoxicity assay at the indicated cold to hot ratio one-half hour before targets were added to the plates. Anti-CD3 (OKT3, Ortho Diagnostics) or control IgG was added to the cytotoxicity assay at a final concentration of 40 µg/ml.

Mixed human/porcine culture conditions

Two x 10^6 porcine stimulator cells were treated with 50μg/ml mitomycin-C (Sigma) in 2 ml PBS for 30 minutes in a 37°C humidified incubator with 5% CO_2. The stimulators were washed 3 times in PBS. Two x 10^6 human effector cells were cocultured with the porcine stimulator cells in a 24 well flat-bottom plate in Aim-V media for 6 days in a 37°C humidified incubator with 5% CO_2. Effectors were harvested, washed and used in a cytotoxicity assay. Anti-CD25 (Pharmingen, azide-free) was added to the mixed culture at a final concentration of 15 μg/ml.

Magnetic bead cell separations and FACS analysis

Human PBLs were prepared from Ficoll/Hypaque gradients. In order to isolate a CD56-enriched population, 2 x 10^7 PBLs were incubated with 10 μg/ml anti-CD3 in 1 ml PBS at 4°C on a rotating platform. After 30 minutes, the cells were washed 3 times with PBS and 4 x 10^8 goat anti-mouse coated magnetic beads (Dynal) in 1 ml PBS with 1% heat inactivated FBS were added. The mixture was incubated as before for an additional hour. For a CD56-depleted population, the same procedure was performed but instead of anti-CD3, anti-CD56 antibody (Pharmingen, B159.5) was added. Purified populations were analyzed by flow cytometry (Coligan, J.E. et al. (1991) Current Protocols in Immunology Vol. 2, Coico, R. ed. (John Wiley and Sons, New York) ch. 7) with primary antibodies, anti-CD3 and anti-CD56. FITC-conjugated goat anti-mouse antibody was used as the secondary antibody. Control incubations were carried out without primary antibody.

IL-2 detection

Supernatants (100 μl) from mixed human/porcine cultures were collected each day after the start of the cultures. IL-2 was detected in these supernatants by ELISA (Endogen). Anti-CD25 was added at a final concentration of 15 μg/ml to prevent the utilization of IL-2.

EXAMPLE III: PBLS FROM NORMAL HUMAN DONORS HAVE CYTOLYTIC ACTIVITY TOWARD PORCINE PBLS

Human serum contains natural antibodies which have been shown to be toxic to porcine cells and are thought to be responsible for hyperacute rejection of porcine organs (Satake, M. et al. (1993) *Clin. Transplant.* 7:281; Kirk, A.D. et al. (1993) *Transplantation* 56:785; Satake, M. et al. (1994) *Xenotransplantation* 1:24). To differentiate between ADCC and that which is antibody independent killing, lysis of porcine cells by human cells in the presence or absence of human serum was tested. The results of this experiment are shown in Figures 5A-5C. PBLs from normal blood donors were isolated and used as effector cells for cytotoxic activity against ^{51}Cr-labeled porcine PBLs. Figures 5A-5C show that normal

human subjects have anti-porcine cytotoxic activity; human cells were-not lysed in this assay (Figures 5A-5B, JY cells). In particular, Figures 5A-5C show that while non-serum dependent cytotoxicity against porcine cells is present in human PBL preparations, an increase in lysis in the presence of serum was not always apparent. The magnitude of non-serum dependent lysis varied among individuals. Allogeneic target cells were not lysed in these assays.

The ability of human cells to lyse porcine cells does not appear to be limited to some individuals as all human subjects tested (n=20) showed cytotoxicity toward porcine cells. In addition, susceptibility of target cell donor was not restricted to a particular type of pig, as outbred stocks and inbred minipigs were all sensitive targets. To test whether this phenomenon was specific to the human/pig combination, or whether human PBLs had lytic activity against other xenogeneic target cells, mouse (Balb/c) and rat (Sprague/Dawley) spleen cells were labeled with ^{51}Cr and used as targets for human PBLs. While porcine cells were lysed at 100:1 effector to target ratio (25% specific release), rat and mouse cells were not lysed under these conditions. The results from these studies demonstrated that peripheral blood lymphocytes from normal donors have cytolytic activity toward porcine PBLs.

EXAMPLE IV: CYTOTOXIC LYMPHOCYTE RESPONSE BY MIXED CULTURE OF HUMAN LYMPHOCYTES WITH PORCINE CELLS IS NOT MHC-RESTRICTED

The antibody-independent cytolytic activity discussed above could be due to cytotoxic T cells (CTLs), NK cells or other cytolytic cells. To test whether there was an MHC-restricted CTL response by mixed culture of human lymphocytes with porcine cells, mitomycin C-treated PBLs were isolated from an NIH inbred minipig of the *aa* haplotype and cultured with human PBLs. After 6 days, effector function was assessed on ^{51}Cr-labeled target cells from *aa* or *dd* pigs. As shown in Figure 6, human effector cells from the mixed culture lysed porcine target cells equally, regardless of the stimulator haplotype. The lysis of porcine targets was increased after coculture relative to unprimed lysis (Figures 5A-5C). Repeated stimulation of human PBLs with porcine stimulators did not yield MHC-restricted lysis of porcine cells.

The lack of MHC restriction in this assay indicated that an effector cell that did not require priming by MHC-peptide complexes could account for the unprimed lysis observed above. The lysis observed after mixed human-porcine culture is due to cells, e.g., NK cells that do not recognize antigen in the context of MHC.

EXAMPLE V: HUMAN NATURAL KILLER CELLS LYSE PORCINE CELLS

To test whether NK cells were responsible for the lysis of porcine cells, cold target inhibition was used with the human NK cell target, K562. As shown in Figure 7A unlabeled K562 cells inhibited the lysis of porcine cells when freshly isolated human PBLs were used as effectors. The lysis of porcine target cells was not inhibited by the NK-resistant cell line, JY. The ability of K562 cells to inhibit the lysis mediated by the human anti-porcine cytotoxic cells generated in mixed culture was also tested. Figure 7B shows that the cytotoxic cells in the mixed culture are inhibited by K562 cells. These results indicate that NK cells are responsible for the lysis of porcine cells by freshly isolated human cells or human cells cocultured with porcine stimulators.

To directly test whether human NK cells are responsible for lysis of porcine cells, cells expressing CD56, a marker for NK cells, were partially purified from PBL preparations. $CD56^+$ cells were enriched by negative selection with anti-CD3 monoclonal antibody and goat anti-mouse antibody coated magnetic beads as described above. These cells were then used as effectors in the ^{51}Cr release assay with porcine cells as the targets. Table I shows the results of FACS analysis of the enriched NK cell population which indicates a 3-fold purification of $CD56^+$ cells. For comparison, $CD56^+$ cells were depleted by a negative selection scheme with anti-CD56 monoclonal antibody and goat anti-mouse coated magnetic beads. For control purposes, tests were also performed to determine whether the CD56-enriched and depleted populations would be active against K562 cells. Figures 8A-8C illustrate that most of the cytotoxic activity toward porcine cells and K562 cells is present in the CD56-enriched population and not in the CD56-depleted population.

TABLE I: Percent Staining[1] of CD56-enriched and CD56-depleted population

	Control	anti-CD3	anti-CD56
Unfractionated	<1	36	10
$CD56^+$-enriched[2]	<1	15	38
$CD56^+$-depleted	<1	84	<1

1. Percent staining as determined by FACS analysis.

2. Enrichment and depletion of $CD56^+$ cells was performed by negative selection with antibody and magnetic beads.

EXAMPLE VI: CYTOTOXIC HUMAN EFFECTORS RAISED AGAINST PORCINE STIMULATORS ARE PHENOTYPICALLY DISTINCT FROM FRESH HUMAN CYTOTOXIC CELLS

Experiments were conducted to determine whether mixed culture of human PBLs and porcine cells led to changes in the human anti-porcine lytic activity. After mixed culture of human PBLs and porcine stimulators, specific lysis ranged from 70 to 90% at an effector to target ratio of 100 to 1, whereas lysis due to fresh human effectors under these conditions ranged from 8 to 30%. While this increase in activity may be due to the outgrowth of cells during mixed culture, analysis of the mixed culture after 6 days revealed an increase in CD56 staining. Gross changes in the various cell populations ($CD4^+$, $CD8^+$ and $CD56^+$) with and without porcine stimulator cells were also detected.

The increase in lytic activity produced during human/porcine mixed culture is due to an increase in the number of NK cells or their differentiated counterparts, lymphokine activated killer cells (LAK). The results shown in Figures 7A-7B demonstrate that the NK cell target, K562, acts as a cold target inhibitor to human anti-porcine cytotoxicity. Tests were also performed to determine whether the LAK target, Daudi (Biron, C.A. et al. (1989) *New Engl. J. Med.* 320:1731), would act as a cold target inhibitor of this response. Figures 9A-9B show that while K562 cells inhibited the unstimulated human anti-porcine cytotoxicity as well as cytotoxicity after mixed culture, Daudi cells inhibited the cytotoxicity after mixed culture only. This indicates that during the mixed culture, LAK cells develop due to the production of cytokines by the culture.

Since the generation of LAK cells from NK cells is dependent on IL-2, an assessment was made as to whether IL-2 is generated by mixed human/porcine culture. Figure 10 shows that human IL-2 is generated in these cultures. Significant IL-2 was measured only in the presence of anti-IL-2 receptor antibody, anti-CD25, suggesting that in the absence of the antibody, IL-2 is difficult to measure due to consumption by the cells in the culture.

To test whether IL-2 in the mixed cultures is required for the increase in specific lysis, anti-CD25 antibody was added to the cultures to block the utilization of IL-2. Figure 11 shows that in the presence of anti-CD25 the specific lysis of porcine targets is reduced, indicating that the generation of LAK cells in these cultures is at least partially dependent on the utilization of IL-2 by the human lymphocytes.

EXAMPLE VII: PRESENCE OF HUMAN CYTOTOXIC T CELL COMPONENT AMONG HUMAN ANTI-PORCINE CYTOTOXIC CELLS

To measure a CTL response in the human/porcine mixed culture which might be obscured by a strong NK component, a test was performed to determine whether removal of $CD56^+$ cells would reveal a T cell response. Human PBLs were depleted of $CD56^+$ cells and

put into culture with mitomycin C-treated porcine PBLs. Cells were harvested on day 6 and the depletion of $CD56^+$ cells was repeated. The cells recovered were then used as effectors in the ^{51}Cr release assay against porcine PBL targets. Figure 12 shows that cytotoxic activity is detected in these cultures after NK cell depletion. This activity is partially blocked by anti-CD3 antibodies (B). The cytotoxicity remaining is not inhibitable with cold K562 cells (D) which suggests that it is not due to NK cells. Thus, the T cell component of the human anti-porcine response is measurable but appears to be overwhelmed by NK mediated lysis.

As shown in Examples III-VII, freshly isolated human PBLs have the ability to lyse porcine cells. In addition, while antibody-mediated mechanisms account for variable levels of cytotoxicity, reproducible killing, in the absence of human serum is attributable to natural killer cells. As described above, the cell line, K562, an established target for human NK cells, acted as a cold target inhibitor in the human anti-porcine cytotoxicity assay. In addition, when the human PBL population was enriched for $CD56^+$ cells, anti-porcine cytotoxicity increased, as did NK cell activity. Depleting the $CD56^+$ cells reduced significantly the anti-porcine lytic activity in these populations. Mixed culture of human and porcine cells prior to the cytotoxicity assay increased the level of anti-porcine cytotoxicity. This activity did not appear to be due to T cells as it was not MHC restricted. IL-2 was generated in these cultures and appeared to be required for the increase in anti-porcine cytotoxicity. IL-2 generated in the human/porcine mixed culture leads to the differentiation of NK cells to LAK cells. Cold target inhibition studies confirmed that LAK cells were generated in the mixed culture. When NK cells were removed from human/porcine mixed cultures, a T cell component to the human anti-porcine cytotoxicity was detected. Therefore, the human anti-porcine cellular cytotoxic response is due to multiple cell types that include T cells in addition to NK and LAK cells.

Other Embodiments

Other embodiments are within the scope of the invention and the following claims. For example, in another embodiment, cells which are administered to a subject according to the methods of the invention are present within a tissue or organ. When cells are within a tissue, antigens on the surface of the cells (e.g., MHC class I antigens) can be altered by contacting the entire tissue with a molecule (e.g., antibody) which binds to the antigen (e.g., incubating the tissue in a solution containing the molecule which binds the antigen). Alternatively, when a cell is within an organ, antigens on the surface of the cells (e.g., MHC class I antigens) can be altered by perfusing the organ with a solution containing a molecule (e.g. antibody) which binds to the antigen. An organ can be perfused with a solution containing the molecule using conventional techniques for organ perfusion.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

5

CLAIMS

1. A cell which has at least one antigen on the cell surface which stimulates an immune response against the cell when the cell is transplanted into a recipient subject, wherein the at least one antigen on the cell surface is altered such that natural killer cell-mediated rejection of the cell is inhibited.

2. The cell of claim 1, which is a porcine cell.

3. The cell of claim 3, which is selected from the group consisting of an endothelial cell, an hepatocyte, a pancreatic islet cell, a skeletal myocyte, a skeletal myoblast, a cardiac myocyte, a cardiac myoblast, a fibroblast, an epithelial cell, a neural cell, a bone marrow cell, an hematopoietic cell, and a lymphoid cell.

4. The cell of claim 1, wherein the recipient subject is a xenogeneic recipient subject.

5. The cell of claim 1, which is contacted with at least one molecule which binds to the antigen.

6. The cell of claim 5, wherein the at least one molecule which binds to the antigen is an antibody, or fragment or derivative thereof, which binds to the antigen but does not activate complement or induce lysis of the cell.

7. The cell of claim 6, wherein the antibody, fragment or derivative thereof, is an $F(ab')_2$ fragment.

8. The cell of claim 1, wherein the at least one antigen is an MHC class I antigen.

9. The cell of claim 8, which is contacted with at least one anti-MHC class I antibody, or fragment or derivative thereof, which binds to the MHC class I antigen but does not activate complement or induce lysis of the cell.

10. The cell of claim 9, wherein the at least one anti-MHC class I antibody is an anti-MHC class I $F(ab')_2$ fragment.

11. The cell of claim 8, wherein the anti-MHC class I $F(ab')_2$ fragment is a F(ab')2 fragment of a monoclonal antibody selected from the group consisting of W6/32, PT85, a

monoclonal antibody which binds an epitope bound by W6/32 and a monoclonal antibody which binds an epitope bound by PT85.

12. The cell of claim 1, which is within a tissue or an organ.

13. A porcine cell which has an MHC class I antigen on the cell surface, the MHC class I antigen having bound thereto an anti-MHC class I antibody, or fragment or derivative thereof, wherein, upon transplantation of the porcine cell into a xenogeneic recipient, natural killer cell-mediated rejection of the cell is inhibited.

14. The cell of claim 13, wherein the at least one anti-MHC class I antibody is an anti-MHC class I $F(ab')_2$ fragment.

15. The cell of claim 14, wherein the anti-MHC class I $F(ab')_2$ fragment is a F(ab')2 fragment of a monoclonal antibody selected from the group consisting of W6/32, PT85, a monoclonal antibody which binds an epitope bound by W6/32 and a monoclonal antibody which binds an epitope bound by PT85.

16. The porcine cell of claim 13, which is selected from the group consisting of an endothelial cell, an hepatocyte, a pancreatic islet cell, a skeletal myocyte, a skeletal myoblast, a cardiac myocyte, a cardiac myoblast, a fibroblast, an epithelial cell, a neural cell, a bone marrow cell, an hematopoietic cell, and a lymphoid cell.

17. A method for reducing the immunogenicity of a cell for transplantation into a recipient subject, wherein the cell has at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject, comprising contacting the cell with at least one molecule which binds to the antigen on the cell surface such that when the cell is transplanted into a recipient subject, natural killer cell-mediated rejection of the cell is inhibited.

18. The method of claim 17, wherein the at least one molecule which binds to the antigen is an antibody, or fragment or derivative thereof, which binds to the antigen but does not activate complement or induce lysis of the cell.

19. The method of claim 18, wherein the antibody, or fragment or derivative thereof, is an $F(ab')_2$ fragment.

20. The method of claim 17, wherein at least one antigen is an MHC class I antigen.

21. The method of claim 20, wherein the cell is contacted with at least one anti-MHC class I antibody, or fragment or derivative thereof, which binds to the MHC class I antigen but does not activate complement or induce lysis of the cell.

22. The method of claim 21 wherein the at least one anti-MHC class I antibody is an anti-MHC class I $F(ab')_2$ fragment.

23. The method of claim 22, wherein the anti-MHC class I $F(ab')_2$ fragment is a $F(ab')_2$ fragment of a monoclonal antibody selected from the group consisting of W6/32, PT85, a monoclonal antibody which binds an epitope bound by W6/32 and a monoclonal antibody which binds an epitope bound by PT85.

24. The method of claim 17, wherein the cell is a porcine cell and the recipient subject is a human.

25. The method of claim 23, wherein the porcine cell is selected from the group consisting of an endothelial cell, an hepatocyte, a pancreatic islet cell, a skeletal myocyte, a skeletal myoblast, a cardiac myocyte, a cardiac myoblast, a fibroblast, an epithelial cell, a neural cell, a bone marrow cell, an hematopoietic cell, and a lymphoid cell.

26. A method for reducing the immunogenicity of a porcine cell for transplantation into a recipient subject, wherein the porcine cell has an MHC Class I antigen on the porcine cell surface, comprising contacting the porcine cell with an anti-MHC class I antibody, or fragment or derivative thereof, such that when the porcine cell is transplanted into a recipient subject, natural killer cell-mediated rejection of the porcine cell is inhibited.

27. A method for transplanting a cell into a recipient subject such that rejection of the cell by the recipient subject is inhibited, comprising administering to the subject a cell having at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject, wherein the at least one antigen on the cell surface is altered prior to transplantation to inhibit natural killer cell-mediated rejection of the cell by the recipient subject.

28. The method of claim 27, wherein the recipient subject is a xenogeneic recipient subject.

29. The method of claim 27, wherein the cell is contacted with at least one molecule which binds to the antigen.

30. The method of claim 29, wherein the at least one molecule which binds to the antigen is an antibody, or fragment or derivative thereof, which binds to the antigen but does not activate complement or induce lysis of the cell.

31. The method of claim 30, wherein the antibody, or fragment or derivative thereof, is an $F(ab')_2$ fragment.

32. The method of claim 27, wherein at least one antigen is an MHC class I antigen.

33. The method of claim 32, wherein the cell is contacted with at least one anti-MHC class I antibody, or fragment or derivative thereof, which binds to the MHC class I antigen but does not activate complement or induce lysis of the cell.

34. The method of claim 33, wherein the at least one anti-MHC class I antibody is an anti-MHC class I $F(ab')_2$ fragment.

35. The method of claim 34, wherein the anti-MHC class I $F(ab')_2$ fragment is a $F(ab')_2$ fragment of a monoclonal antibody selected from the group consisting of W6/32, PT85, a monoclonal antibody which binds an epitope bound by W6/32 and a monoclonal antibody which binds an epitope bound by PT85.

36. The method of claim 27, wherein the cell is a porcine cell.

37. The method of claim 36, wherein the porcine cell is selected from a group consisting of an endothelial cell, an hepatocyte, a pancreatic islet cell, a skeletal myocyte, a skeletal myoblast, a cardiac myocyte, a cardiac myoblast, a fibroblast, an epithelial cell, a neural cell, a bone marrow cell, an hematopoietic cell, and a lymphoid cell.

38. A method for transplanting a porcine cell into a xenogeneic recipient subject such that rejection of the porcine cell by the recipient subject is inhibited, comprising: administering to the subject a porcine cell having an MHC Class I antigen on the porcine cell surface, wherein, prior to transplantation, an anti-MHC Class I antibody is bound to the MHC Class I antigen to inhibit natural killer cell-mediated rejection of the cell by the recipient subject.

39. A cell which has at least one antigen on the cell surface which stimulates an immune response against the cell when the cell is transplanted into a recipient subject,

wherein the at least one antigen on the cell surface is altered such that lymphokine activated killer cell-mediated rejection of the cell is inhibited.

40. The cell of claim 39, which is a porcine cell.

41. A method for reducing the immunogenicity of a cell for transplantation into a recipient subject, wherein the cell has at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject, comprising contacting the cell with at least one molecule which binds to the antigen on the cell surface such that when the cell is transplanted into a recipient subject, lymphokine activated killer cell-mediated rejection of the cell is inhibited.

42. A method for reducing the immunogenicity of a porcine cell for transplantation into a recipient subject, wherein the porcine cell has an MHC Class I antigen on the porcine cell surface, comprising contacting the porcine cell with an anti-MHC class I antibody, or fragment or derivative thereof, such that when the porcine cell is transplanted into a recipient subject, lymphokine activated killer cell-mediated rejection of the porcine cell is inhibited.

43. A method for transplanting a cell into a recipient subject such that rejection of the cell by the recipient subject is inhibited, comprising administering to the subject a cell having at least one antigen on the cell surface which stimulates an immune response against the cell in the recipient subject, wherein the at least one antigen on the cell surface is altered prior to transplantation to inhibit lymphokine activated killer cell-mediated rejection of the cell by the recipient subject.

44. A method for transplanting a porcine cell into a xenogeneic recipient subject such that rejection of the porcine cell by the recipient subject is inhibited, comprising: administering to the subject a porcine cell having an MHC Class I antigen on the porcine cell surface, wherein, prior to transplantation, an anti-MHC Class I antibody is bound to the MHC Class I antigen to inhibit lymphokine activated killer cell-mediated rejection of the cell by the recipient subject.

FIG. 1

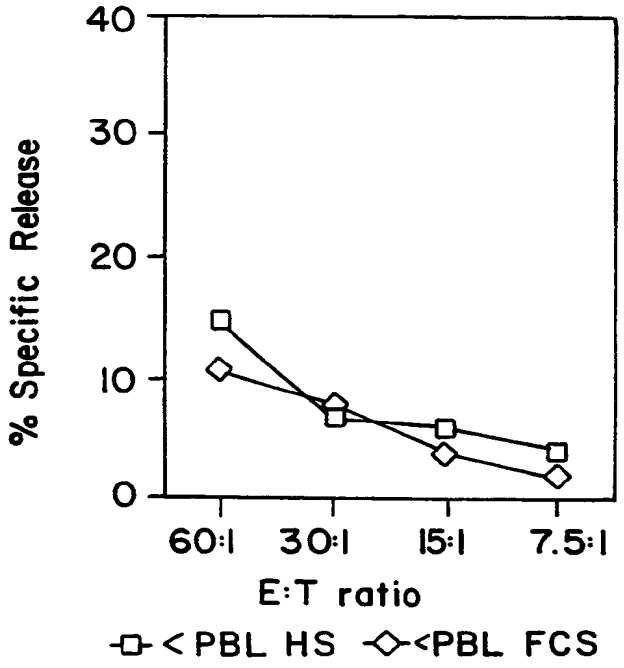


FIG. 2

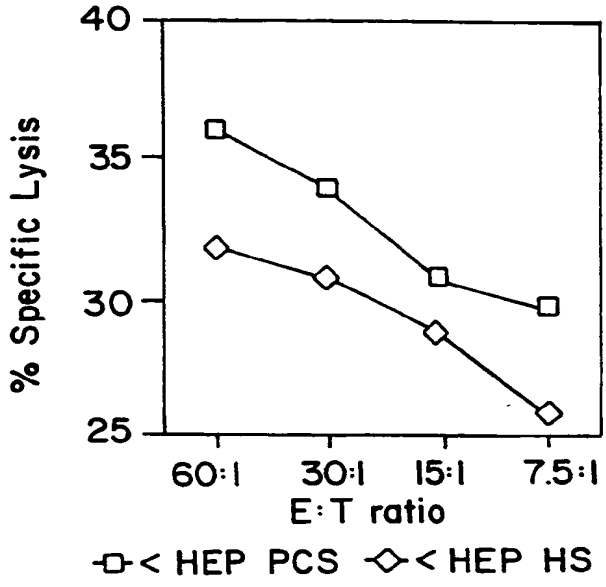
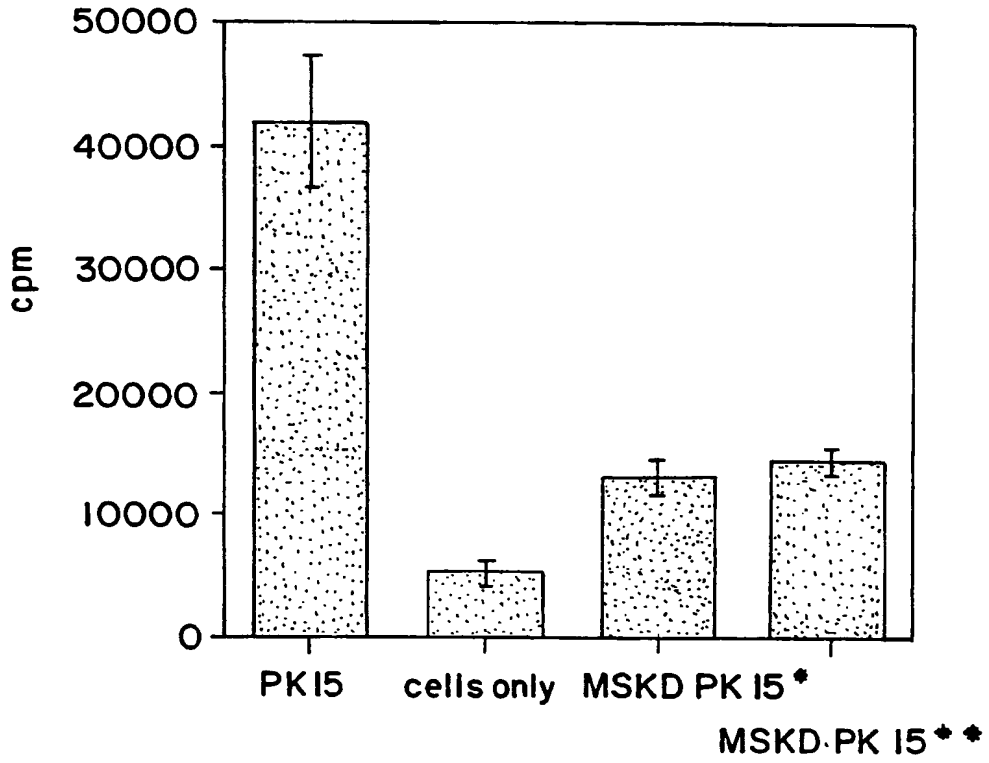


FIG.3



* Masked at 4°c for 2 hours

** masked at 37°c for 16 hours

cpm

FIG. 4

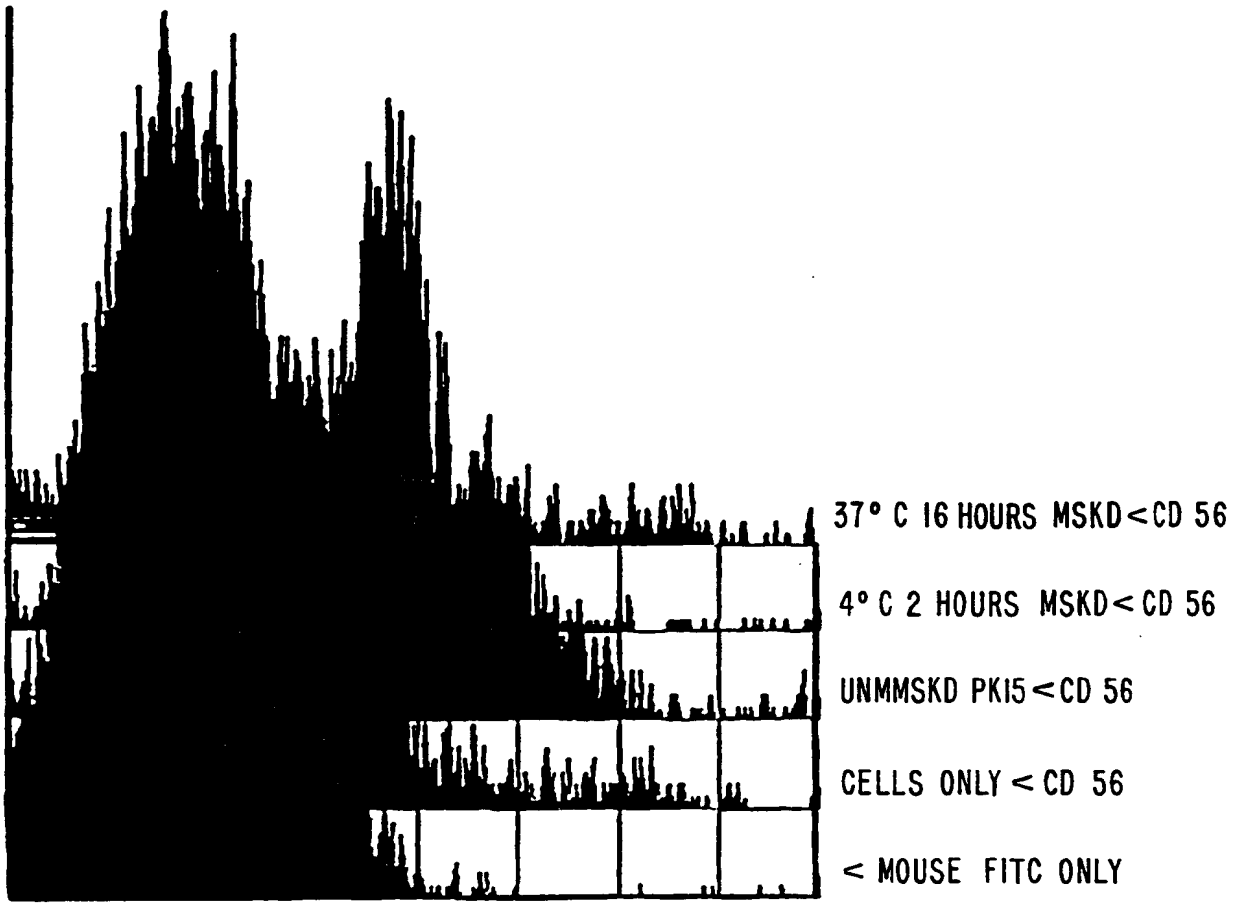


FIG. 5A

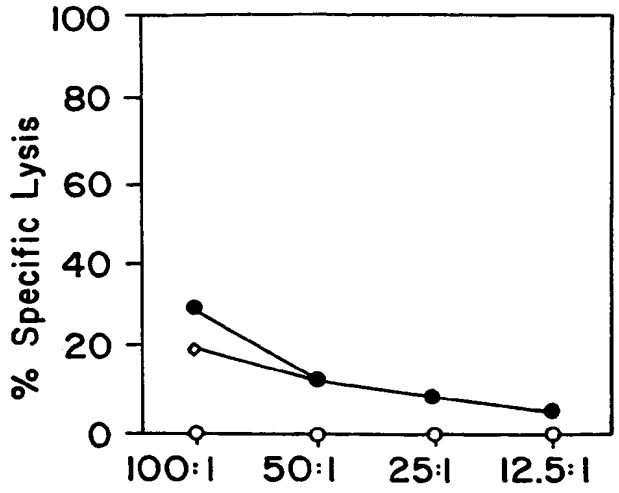


FIG. 5B

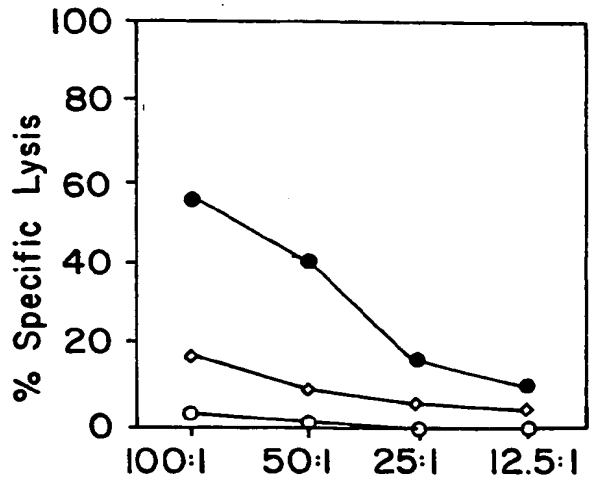


FIG. 5C

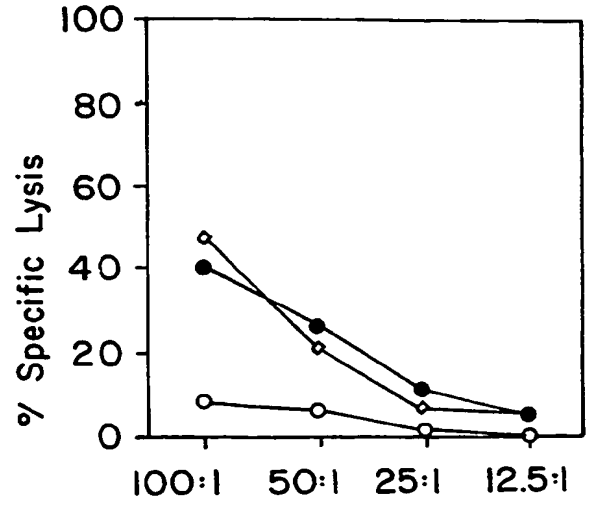


FIG. 6

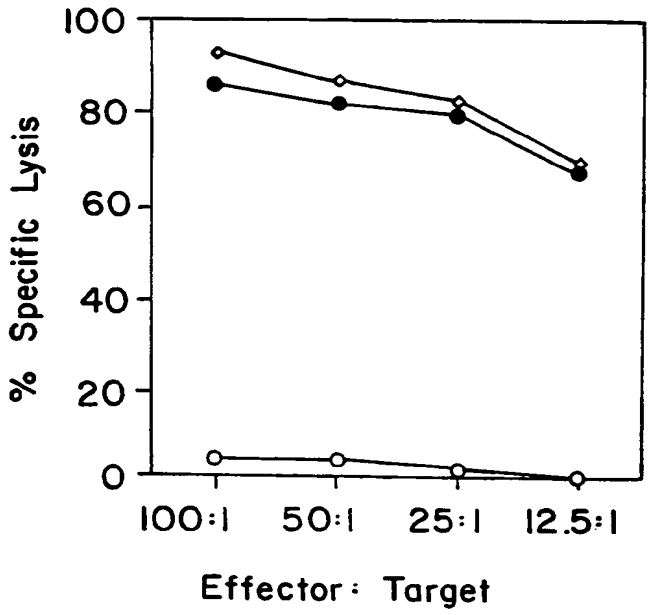


FIG.7A

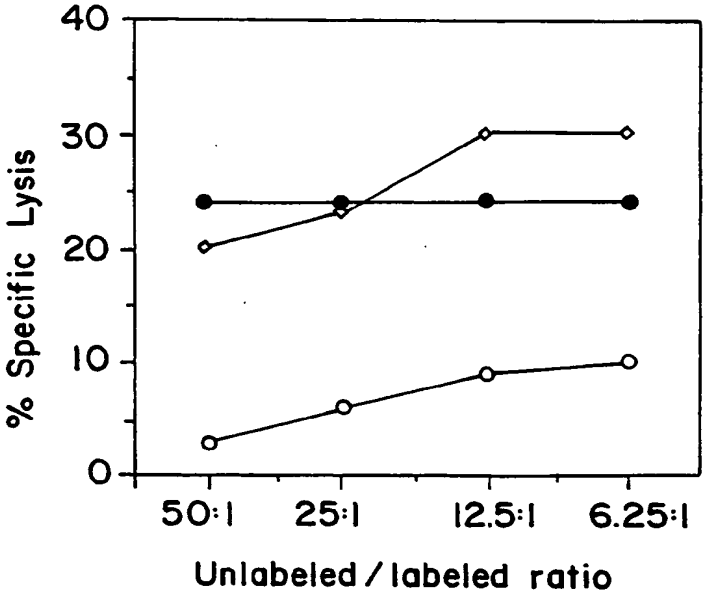


FIG.7B

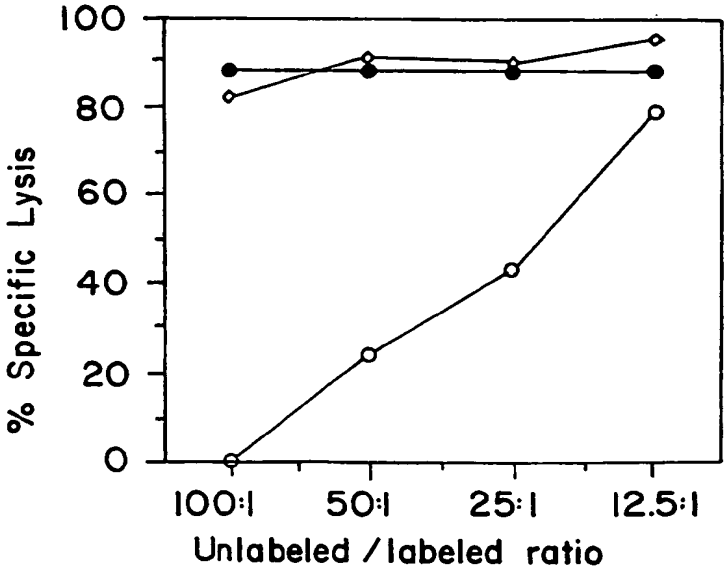


FIG.8A

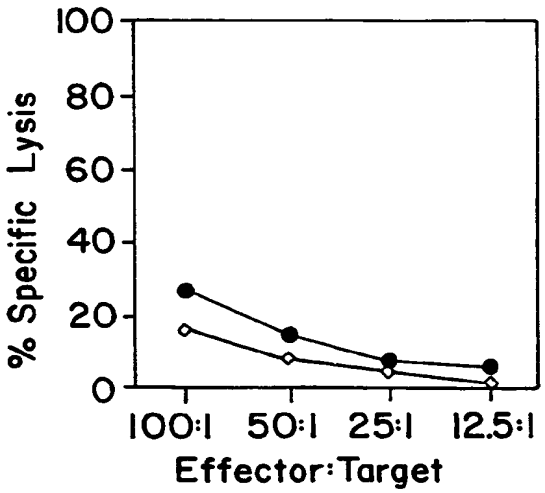


FIG.8B

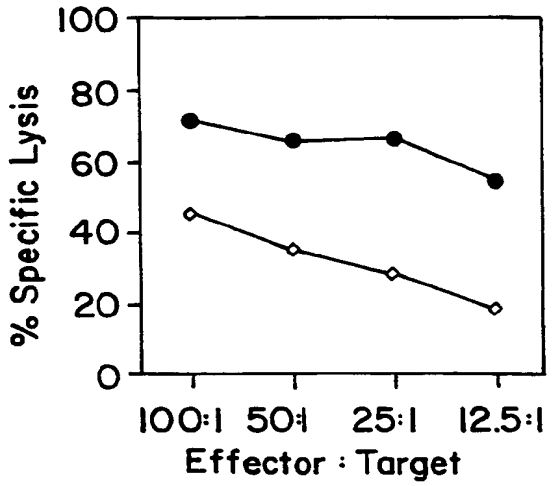


FIG.8C

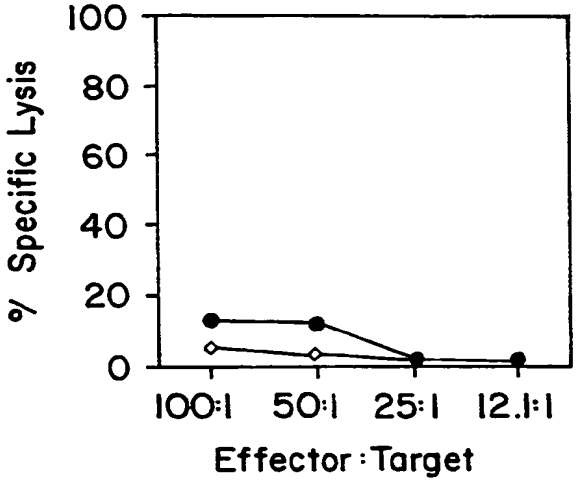


FIG.9A

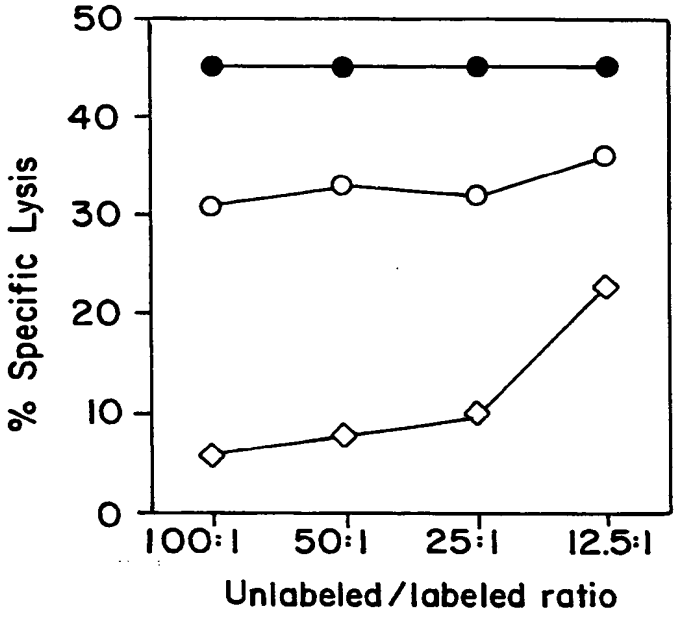


FIG.9B

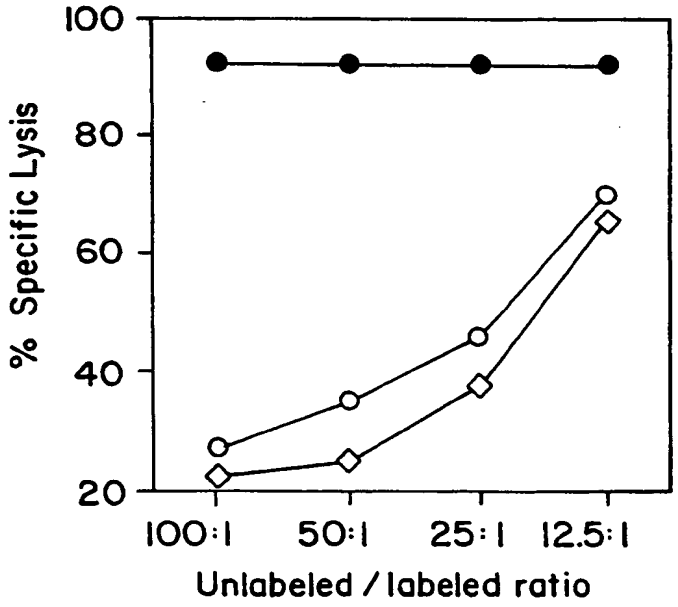


FIG. 10

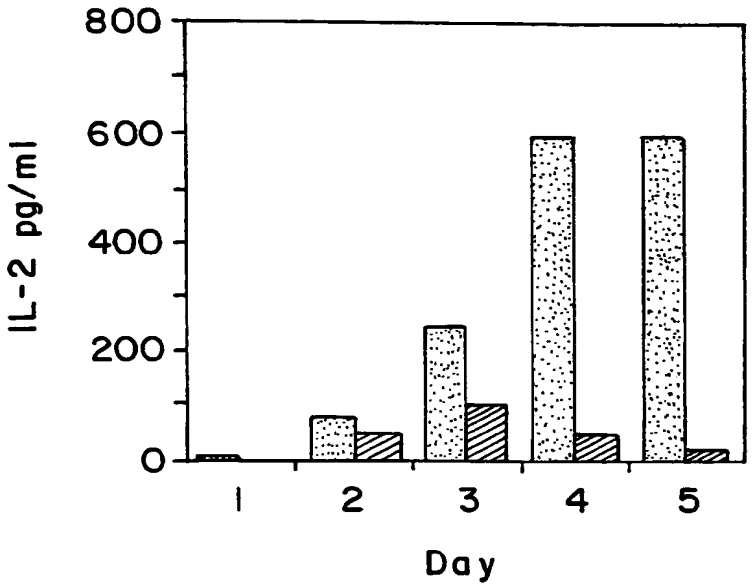


FIG. 11

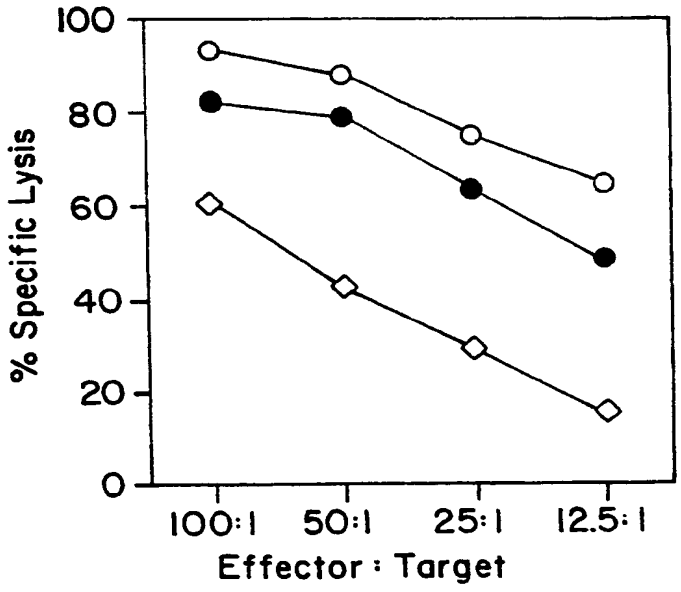
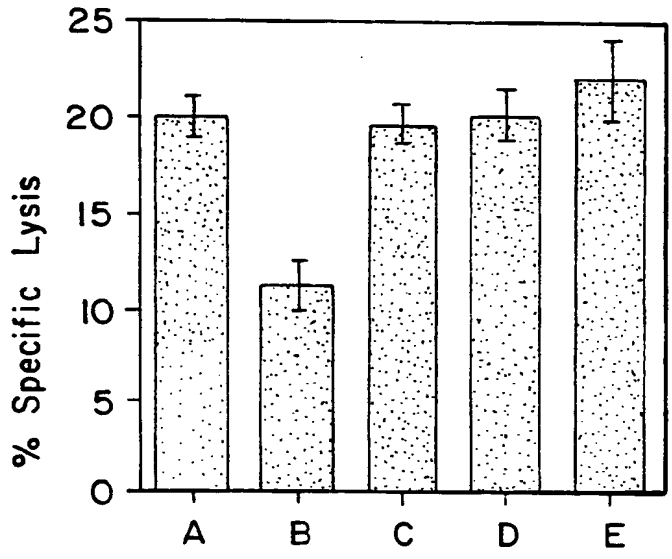


FIG.12



INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/05519

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N5/06 A61K35/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 04033 (THE GENERAL HOSPITAL CORPORATION) 19 March 1992 cited in the application see the whole document ---	1-44
A	SCIENCE, vol. 252, no. 5013, 21 June 1991, WASHINGTON, DC, USA, pages 1700-1702, XP002011037 D. FAUSTMAN ET AL.: "Prevention of xenograft rejection by masking donor HLA class I antigens." cited in the application see the whole document --- -/--	1-44

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
20 August 1996	28.08.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nooij, F

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/05519

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TRANSPLANTATION, vol. 55, no. 4, April 1993, BALTIMORE, MD, USA, pages 940-946, XP000579608 X. LI ET AL.: "Use of donor beta2-microglobulin-deficient transgenic mouse liver cells for isografts, allografts, and xenografts." see abstract	1-44
A	MOLECULAR IMMUNOLOGY, vol. 26, no. 9, September 1989, OXFORD, GB, pages 883-895, XP000579606 J. HILDRETH ET AL.: "Monoclonal antibodies against porcine LFA-1: species cross-reactivity and functional effects of beta-subunit-specific antibodies." see abstract	1-44
P,A	MOLECULAR IMMUNOLOGY, vol. 32, no. 11, August 1995, OXFORD, GB, pages 789-794, XP000578173 M. SALCEDO ET AL.: "Altered MHC class I presented peptide repertoire is not sufficient to induce NK cell mediated F1-hybrid resistance." see abstract see introduction	1-44

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/ 05519

B x I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 27-38, 43,44 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.